Studies on the availability of soil phosphate to plants

By

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CONTENTS

SUMMARY

The work of this thesis is concerned with the study of some aspects of phosphorus dynamics in soils varying in physico-chemical characteristics. Chapter 1 presents a general introduction and short review of some aspects of phosphorus behaviour in soils. The main emphasis has been given to phosphate availability to plants.

Chapter 2 deals with the analytical techniques used in the present study. A short description of the sites of soil sampling has been given. Some physico-chemical properties of the soils have also been described.

Chapter 3 is about the P status of 14 soils. Six conventional procedures were adopted to measure all types of extractable phosphate in the soils studied. The effectiveness of various extractants was assessed through their relationship with one another. All of the extractants showed better relationship in slightly acidic soils than in slightly alkaline soils. Bray-I (acid ammonium fluoride) extracted the highest P in the former soils, but its performance was extraordinary poor in the latter soils. Bray-I, acetic acid and anion exchange resin extractable P was not significantly correlated with other extractants in the soils of above pH 6.2. However, their relationship with other extractants was much better in soils of pH below 6.2. Olsen 0.5M sodium bicarbonate extractable P was significantly correlated with extractable P of sodium

acetate ($r=0.81^{***}$), ammonium acetate ($r=0.75^{**}$) and anion exchange resin ($r=0.73^{**}$). Anion exchange resin, which is regarded as being analogous to plant available P showed more or less similar behaviour with other extractants. Acetic acid, sodium acetate and ammonium acetate had very highly significant correlation among themselves in all soils. The phosphate extracted by various extractants depends on the relative amount of calcium, iron and aluminium phosphate in a soil and the nature and composition of an extractant.

Phosphate adsorption study of 14 soils has been described in Chapter 4. Adsorption isotherms of all soils were made with standard P concentration points of 0.5, 1, 5, 10, 20, 30, 40, 50, 70 and 100 µg P cm^{-3}. All the soils showed different behaviour towards P adsorption. The P adsorption data conformed well to the Freundlich equation in most of the soils, while only in some soils to the Langmuir equation. Langmuir P adsorption maximum and bonding energy constant for all soils were calculated. P adsorption maximum was significantly related to pH ($r=-0.63^{*}$) and oxalate extractable Fe ($r=0.82^{***}$) and Al ($r=0.61^{*}$). When a few points at the top for the Freundlich plot or at the bottom for the Langmuir plot were excluded from calculation, the R^2 values were greatly improved.

Chapter 5 is concerned with the detailed studies of P adsorption and desorption in 3 soils. Keeping in view the errors assessed in the initial P adsorption and desorption

studies, a series of experiments were run and errors were measured. By adopting the improved experimental technique, the P adsorption/desorption isotherms were in better order. However, the top point at the adsorption and desorption isotherms did not meet as they should. The cause of the gap between the two points was investigated through the effect of time of shaking and soil sterilization. It was observed that P adsorption increased with time. There was no significant instantaneous P desorption except in Dunlop A soil. All the soil sterilants affected the P status of soils except sodium azide and toluene.

Chapter 6 deals with the pot experiment on ryegrass (Lolium perenne). The effect of plant growth on extractable and adsorbed P and enzymic activities in 5 soils is discussed. The results showed significantly highest dry matter(tops) yield in Darvel A and Midelney (grass) soils. P content of leaves was highest in the Midelney soil. The $NaHCO_3$ extractable P before planting was significantly correlated with the dry matter (tops) yield ($r=0.90^*$) and with P uptake ($r=0.89^*$). The P uptake in leaves was also significantly correlated with the dry matter yield ($r=0.91^*$). The extractable P by 3 extractants in grassed soils was significantly reduced than nongrassed soils and in some soils before planting. The effect of plant growth was observed on P adsorption isotherms. The regression analysis of the Langmuir equations obtained from P adsorbed before planting, non-grassed and grassed soils showed

significant differences only in Midelney (Grass) and Dreghorn A soils. The plant growth also revealed variable effects on enzymic activities in before planting, grassed and non-grassed soils.

In chapter 7 a brief account of the experimental results and the general conclusions were made. Further development of this work is also suggested.

CHAPTER 1

SOIL PHOSPHATE AVAILABILITY TO PLANTS

1.1 Introduction

The importance of phosphorus in crop production is well documented (Yerokun, 1987; Holford, 1989). Therefore it is common practice for farmers to include phosphorus in their fertilizer management programme. Phosphorus is the second most important plant nutrient (after nitrogen) for agricultural production in most regions of the world. In contrast to nitrogen, whose supply from the atmosphere is virtually unlimited by natural cycling, phosphorus is recycled in negligible amounts. Phosphorus makes up about 0.12 percent of the earth's crust (Cathcart, 1980). Reserves of the phosphorus are finite, and their increasing depletion will probably be the primary limitation to the continuation of existing levels of life on the earth. The efficient use of phosphorus in soils, therefore, cannot be over-emphasized.

The practice of continuous fertilization has resulted in the establishment of high P levels in many regions of intensive agriculture. This can bring about concerns about soil nutrient imbalances as well as for the environment. On the other hand, such residual P may be as effective as fresh P application for crop production (Mattingly, 1971).

Phosphorus is very chemically reactive, forming at least 172 minerals in nature (Holford, 1989). Thermodynamic principles dictate that phosphate compounds will tend to transform to increasingly stable (unavailable to plants) forms with the passage of time. Consequently, phosphate in soil is the most immobile, inaccessible and unavailable of all nutrient elements. These characteristics make phosphate resistant to leaching in most soils, but at the same time they cause widespread deficiency of phosphate for agricultural production.

The significant role of phosphorus in building up and sustaining land fertility has long been recognized by agricultural workers and farmers all over the world. In spite of marked advancement in our knowledge about phosphorus behaviour in soil, our understanding about phosphorus dynamics in the soil plant system is still far from complete. The trend in the past few years has been to investigate the physical chemistry of phosphate adsorption onto surfaces of soil components, which will lead to ultimate understanding of its behaviour, physical and chemical interactions and transformations, and the factors that affect the rate and direction of such transformations. The experimental approaches and techniques developed to evaluate the behaviour of soil phosphate include soil test methods, soil fertilizer reaction products, Q/I measurements, adsorption/desorption isotherms, phosphorus

transformation processes and the role of phosphorus in soil-plant relationships.

The dynamics of phosphorus transformations in the soil system and its fixation and release have been the subject of numerous research investigations, but in actual practice the most pertinent issue is to know how much of the phosphorus in soil can be made available to growing plants.

1.2 Forms of soil phosphorus

The various forms of phosphorus in soils and their dynamic interrelationships are summarized in Fig 1.1. Soil phosphorus can be classed generally as inorganic and organic , depending upon the nature of compounds in which it occurs. The inorganic forms of phosphorus in soil are frequently greater than that of organic phosphorus. An exception to this rule would, of course, be the phosphorus contained in predominantly organic soils. In addition, the organic phosphorus content of mineral soils is usually higher in the surface horizon than it is in the subsoil because of the accumulation of organic matter in the upper part of the soil profile.

1.2.1 Inorganic soil phosphorus

The primary and secondary minerals, which are crystalline and have been identified by optical and X-ray methods, account for only a small portion of the inorganic

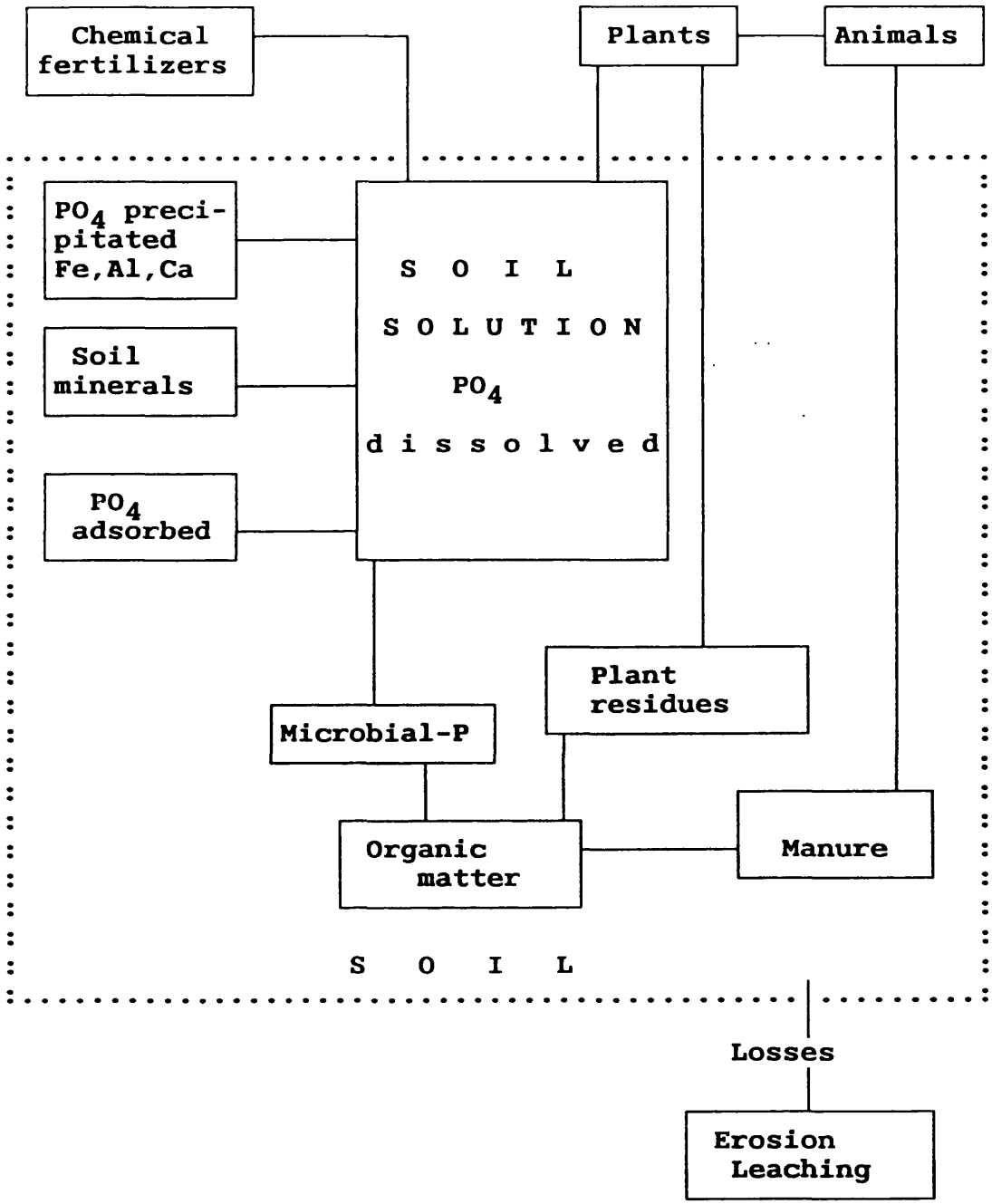


Fig 1.1 Dynamics of phosphate in soil

phosphate in soil. A voluminous literature is available on the distribution of various forms of inorganic phosphorus. There are certain soil properties which regulate the distribution of inorganic forms of phosphorus. These are pH, soluble and exchangeable P-reactive cations (iron, aluminium, calcium, magnesium), and the nature and surface area of soil particles. In all soils, P will occur in adsorbed phases on the surfaces of iron and aluminium hydrous oxides and other clay minerals. However, in soils most of the inorganic phosphorus occurs in the clay fraction as salts of orthophosphoric acid. Attempts have been made to classify inorganic soil phosphorus into different compounds according to their extractability in various reagents (Chang and Jackson, 1957). However, such a classification appears to be arbitrary and does not reveal their true nature. A good deal of knowledge concerning the nature of soil phosphorus is based on solubility product principles. Phosphorus forms difficulty soluble compounds with iron and aluminium at low pH, more soluble compounds with calcium and magnesium at pH values near neutrality and difficulty soluble compounds with calcium at higher pH values (Tisdale et al., 1985). There is wide range in solubility of these various phosphate compounds and their availability to crops is usually the greatest within the pH range of about 6 to 7 for most agricultural soils.

The inorganic phosphorus compounds are mainly divided into two groups: (i) Phosphorus compounds of

calcium and magnesium (ii) phosphorus compounds of iron and aluminium.

1.2.1.1 Calcium and magnesium phosphates

These occurs in soils in several forms, the most important compounds are given:

1. $Ca(H_2PO_4)_2.H_2O$, monocalcium phosphate, which is the water-soluble component of superphosphate and reacts in soil to form less soluble products.

2. $CaHPO_4.2H_2O$ and $CaHPO_4$, dicalcium phosphate, both hydrated and the unhydrated forms are slightly soluble in water.

3. $Ca_8H_2(PO_4)_6.5H_2O$, Octacalcium phosphate.

4. $Ca_3(PO_4)_2$, tricalcium phosphate

5. $Ca_{10}(PO_4)_6(OH)_2$, hydroxyapatite and $Ca_{10}(PO_4)_6F_2$, fluorapatite and substituted apatites.

6. $MgNH_4PO_4.6H_2O$, struvite, alkaline water-soluble

Dicalcium phosphate, octacalcium phosphate and hydroxyapatite are the principal crystalline phosphates that have been identified in soil (Tisdale et al., 1985). The native phosphorus in soils originated largely from disintegration of rocks containing the mineral apatite, $Ca_{10}(PO_4)_6(F,Cl,OH)_2$. Apatite has also been reported as a common soil mineral by Shipp and Matelski (1960). Hydroxyapatite has been reported to be a stable form over

a wide range of pH (Larsen, 1967). The apatite in its primary form has little or no significance in supplying phosphate to plants, because of very low solubility and rate of solubilization (Wild, 1988). The phosphorus concentration in calcareous soils does not correspond to any one mineral species. It may be controlled by octacalcium phosphate in some soils or by hydroxyapatite in other soils. The hydroxyapatite in soil invariably contains some carbonate ions, which makes it chemically more reactive. The presence of octacalcium phosphate has been reported in soils which have been limed and fertilized with phosphates (Webber and Mattingly, 1970). Baifan and Yichu (1989) suggested a systematic fractionation scheme for inorganic phosphates in calcareous soils, in which they classified calcium phosphate into dicalcium phosphate, octacalcium phosphate and apatite types. They also suggested that these forms are interchangeable. Some other workers reported the fractionation of inorganic phosphorus in the calcareous soils as a series of calcium phosphates with complex physico-chemical reactions and different availability to plant growth (Williams et al., 1967 and 1971; Syers et al., 1972 and Hooker et al., 1980).

The availability of P from various inorganic compounds were compared by Tisdale et al.(1985). It was revealed that there was the highest P availability from struvite ($MgNH_4PO_4.6H_2O$) for oats, as compared to mono- or dicalcium phosphate. In soils containing large amounts

of active magnesium, a number of insoluble magnesium phosphate compounds such as dimagnesium phosphate trihydrate, trimagnesium phosphate and struvite may form. However, these magnesium phosphates are more soluble than dicalcium phosphate and octacalcium phosphate.

1.2.1.2 Iron and aluminium phosphates

A number of aluminium and iron phosphate minerals have been reported to occur in soils. The most common aluminium phosphates are wavellite ($Al_3(PO_4)(OH)_3.5H_2O$) and variscite ($AlPO_4.2H_2O$). The occurrence of varisite has been postulated in soil of slight acidity (Lindsay and Moreno, 1960). At higher pH values, variscite dissolves incongruently, whereby a more basic solid phase of aluminium hydroxy phosphate is formed (Taylor and Gurney, 1964). This material probably controls the phosphorus concentration in solution in acid soil by forming a surface complex on variscite. However, in pure systems, where the pH of the equilibrium solution is less than 3.1, the solubility product of variscite controls the phosphorus concentration in solution.

The most common iron phosphate is strengite ($FePO_4.2H_2O$). Chakravarti and Talibudeen (1962) concluded that a compound approximating to the composition of strengite may occur in temperate soils in the pH range of 3.8 to 4.2, whereas in tropical soils strengite coexists with hydrated iron oxide from pH 3.8 to 6.7.

The least soluble compounds at acid pH are variscite and strengite (Tisdale et al., 1985). Strengite is known to crystallize more rapidly when the iron phosphate is formed. The less crystalline aluminium phosphate has greater surface area which is more favourable for release of phosphorus into the soil solution. Under very acid conditions minerals of the variscite-strengite group are precipitated (Wild, 1988).

1.2.2. Organic soil phosphorus

The amounts of organically held phosphorus vary greatly between soils. Organic phosphorus content of soil occurs from traces in arid soils to several hundred mg/kg in thick forest soils. It is roughly related to organic matter contents both between soils and within the soil profile. Hence the circumstances that lead to increased soil organic matter also generally lead to increased organic phosphorus contents. The organic phosphorus contents of the soils throughout the world ranges between 7 to 1056 mg/kg soil (Campbell and Racz, 1975).

Most naturally occurring organic forms of phosphorus are esters of orthophosphoric acid and numerous mono- and diesters have been characterized. According to Tisdale et al. (1985) these organic phosphorus esters have been divided into five classes of compounds: inositol phosphates, phospholipids, nucleic acids, nucleotides, and sugar phosphates. The first three are the dominant groups in soils.

It is generally assumed that soil organic phosphorus is derived directly, or after biochemical transformations, from crop and animal residues, in which part of the phosphorus is present in organic compounds. The phosphate added as fertilizer is converted to organic forms in soils under young grassland due to increased root production and leaf decay added to the soils (Wild, 1988). The importance of the mineralisation of organic phosphorus compounds for crop nutrition is probably universal. But it is greatest at high soil temperatures because of high mineralisation rates. Mineralisation of organic P may release important amounts of inorganic P in favourable conditions of moisture, pH and root activity.

1.2.2.1 Phosphatase activity

The phosphatase enzymes play a major role in the mineralisation of organic phosphorus in soil. They are the broad group of enzymes that catalyse the hydrolysis of both esters and anhydrides of phosphoric acid. Phosphatase activity of a soil is due to the combined functioning of the soil microflora and any free enzymes present (Tisdale et al., 1985). The phosphatase enzyme excreted by plant roots could hydrolyse organic phosphorus in soils, thus releasing inorganic phosphorus available to plants (Martin, 1973). Tarafdar and Jungk (1987) reported that phosphate ions may be released from organic compounds through reactions catalysed by

phosphatases. These enzymes are contributed from three components of rhizosphere: soil, microorganisms and plant roots. The enzymes are specific activators in that they combine with their substrates in such a stereospecific fashion that they cause changes in the electronic configuration around certain susceptible bonds (Tabatabai, 1982). Physico-chemical measurements indicate that enzyme-catalyzed reactions have lower activation energy than the uncatalyzed counterparts and therefore, have a faster rates of reactions (Browman and Tabatabai, 1978).

The enzymes are denatured by elevated temperature and extreme pH. Their physico-chemical state and their influence on chemical reactions in soil are markedly dependent on pH, ionic strength, temperature and presence or absence of inhibitors or activators (Tabatabai, 1982). The influence of soil texture and organic matter content on phosphatase activity have also been reported by Tarafdar and Jungk (1987).

In spite of the fact that in some mineral soils one-half to two-thirds of the total phosphorus is organic, this important fraction is usually ignored in the measurements of available soil phosphorus as well as when phosphate nutrition of crop plants is considered.

1.3 Assessment of phosphorus status in soil

The assessment of soil phosphorus is an objective, which can be achieved by various ways and for a variety of reasons. Several soil-test methods have been evolved over the years with the objectives: to classify the soils for the purpose of making fertilizer recommendation; to predict the response to application of fertilizer P; and to provide an index of the amount of P a soil can supply. However, assessment is often attempted for the primary purpose of understanding the fundamental principles that govern soil-phosphate-plant interactions. Most of the test methods, proposed for the assessment of plant available P are based on the extraction procedures, which differ in their nature, concentration, pH of solvents used, the soil:solvent ratio, the extraction time and temperature. These determine the strength of extraction and the extent to which the values can be regarded as reflecting the plant availability of P. Kamprath and Watson (1980) described three criteria for a good soil test: (i) the extractant used should extract all or a proportionate part of the available form or forms of a nutrient from soils with variable properties, (ii) the amount of nutrient extracted should be measured with reasonable accuracy and speed, and (iii) the amount extracted should be correlated with the growth and response of each crop to that nutrient under various soil conditions. The P-test provides a relative estimate of the soil P-status, which is thought to be the ability

of a soil to release P to a crop during growing season; or more specifically, the ability of a soil to release P to a given plant root for a given period under optimum conditions with respect to all other growth factors (Sibbesen, 1983). Apart from the soil P-status, the crop yield response to P-addition depends on other factors, such as the chemical, physical, and biological soil properties, the climate and the type of fertilizer added to soil.

In traditional soil-test methods, crop response is correlated with the fraction of soil P that is extracted by a given procedure. Beside the chemical nature of soil P extracted, understanding of physical and chemical interactions and transformations, and the factors that affect such transformation is necessary.

1.3.1 The effects of P-tests on the soil P

Most P-tests are based on extracting soil with a solution which dissolves a proportion of the solid phase P by the following mechanisms: (i) Increasing the soil-solution ratio, which decreases the solution P concentration and thereby disturbing the equilibrium between solid phase P and solution P. If the extractant is water as in the methods of Sissing (1971) and Paauw (1971) then the activity of P precipitating cations in solution is also decreased (Sibbesen, 1983). (ii) The acid solutions used to extract P like Dyer's reagent (citric acid) and Bray's reagent (HCl + NH_4F) provide

sufficient H^+ ion activity to dissolve calcium phosphates, to some extent aluminium and iron phosphates. The order of greater solubility in acid solutions is Ca-P > Al-P > Fe-P (Thomas and Peaslee, 1973). (iii) Extracting solutions containing OH^- ions extract P from Al-P and Fe-P due to the hydrolysis of the Al and Fe. Thus Olsen's reagent (0.5M $NaHCO_3$), which is buffered at pH 8.5 is very effective in extracting Al-P and to some extent Fe-P (Tyner and Davide, 1962). (iv) introducing anions that complex and/or precipitate phosphate precipitating cations such as calcium, aluminium, and ferric ions. In Bray's reagent fluoride is more effective in complexing Al^{3+} ions and releasing P from Al-P (Chang and Jackson, 1957). Calcium is precipitated by F^- ions and therefore the P present in soil as $CaHPO_4$ will be extracted by solutions containing F^- ions (Thomas and Peaslee, 1973). The organic anions, citrate and lactate also complex Al^{3+} ions (Kamprath and Watson, 1980). (v) phosphorus adsorbed on the surfaces of $CaCO_3$ and hydrated oxides of Fe and Al are replaced by other anions such as acetate,lactate and bicarbonate ions in the extracting solution (Olsen et al., 1954). These anions also limit the resorption of dissolved phosphate (Sibbesen, 1983).

In contrast to the plant root, which removes P from the soil solution, these extractants accumulate P in the liquid phase. However, part of the dissolved P is reprecipitated and/or readsobed, because the chemical reagents that break the bonds between phosphate and some

soil constituents at the same time favour formation of other bonds (Sibbesen, 1983). Thus the final P extracted is the result of two opposite reactions.

1.3.2 Soil factors affecting P uptake

According to Kamprath and Watson (1980) the factors affecting the supply of P to plants are the amount of soil P (quantity), the concentration of soil solution P (intensity), and movement of P to roots (diffusion). In any assessment of the available P in soil by chemical tests, one needs to consider the relationship between quantity, intensity, and diffusion, and factors influencing these components of P to plants.

1.3.2.1 Phosphate buffering capacity

The P equilibrium between solid phase and solution phase is characterized by the phosphate buffering capacity of a soil. This is the ability of a soil to maintain its P concentration in solution as P immobilized by soil through various processes or as P is added by fertilization. Thus the availability of soil phosphate is described by both intensive and extensive parameters which are determined by the concentration of phosphorus in soil solution (I) and the quantity of phosphate adsorbed on to the soil solids (Q) respectively. These two parameters determine the buffering capacity of soil (Barrow, 1967; Holford, 1976), which regulates the resistance of I and Q to change when phosphate is added

or desorbed by the system (Holford and Mattingly, 1976a). The buffering capacity of a soil is the slope of the adsorption isotherm at some arbitrary concentration, which may reflect the actual P concentration found in the solution (Beckett and White, 1964), or theoretical P concentration sufficient for plant growth (Ozanne and Shaw, 1967) or the calculated maximum slope at zero concentration (Holford and Mattingly, 1976a). Soils with high phosphate adsorption maxima have higher phosphate buffering capacity than those with low phosphate adsorption maxima (Rajan, 1973). The higher or stronger the buffering capacity, the larger the proportion of P in solid phase relative to solution phase; increasing buffer capacity also lowers the rate of dissolution or desorption of P from the solid phase and vice versa (Holford, 1989). According to Kamprath and Watson (1980) the buffering capacity of acid and neutral soils is a function of the amounts and crystallinity of hydrated oxides of Fe and Al, whereas in calcareous soils the amounts of exchangeable Ca^{2+} and $CaCO_3$ determine the P buffering capacity.

1.3.2.2 Phosphate diffusion in soil

Diffusion of phosphorus through the soil to the roots is the dominant mechanism governing the P-uptake by roots growing in all soils, except those extremely high in phosphorus. Barrow (1989) suggested that phosphate

mostly moves to plant roots by diffusion and it is only the phosphate in the soil solution that is free to move. The plant root reduces the soil-P mainly by absorbing from the adjacent soil solution, which initiates the diffusion of P in the soil solution towards the plant root and the dissolution of solid phase P, termed as the labile pool (Schofield, 1955). The intensity, or the concentration of P in the soil solution, is determined by the percent saturation of the P adsorption capacity (Kamprath and Watson, 1980). The replenishment process, which involves the increase of P concentration in soil solution has been regarded as the primary index of available P (Schofield, 1955; Holford, 1989). At any particular concentration of P, the higher the buffer capacity the greater the replenishment. Phosphate moves from a point of higher concentration to a point of lower concentration when a concentration gradient exists. The concentration gradient in soil across the root surfaces is an important factor influencing P diffusion (Kamprath and Watson, 1980). Soil texture is another factor affecting diffusion of P (Olsen and Watanabe, 1963). As the clay content increases, the diffusion coefficients increase due to a decrease in tortuosity and an increase in buffering capacity. The diffusion of phosphate persists until the equilibrium is established. Since the diffusion of phosphorus occurs essentially in the liquid phase and an individual phosphate ion spends a relatively short time in this phase, the diffusion coefficient of

phosphorus in the soil solution will be different from that in free solution. Diffusion coefficient of phosphate through soil is in range of 10^{-8}-10^{-11} cm^2s^{-1}. Diffusion through the solid phase is extremely slow and the phosphate ion, being negatively charged, would not be likely to diffuse along the negatively charged surfaces of soil particles. Fitter (1992) stated that a phosphate ion normally moves less than a millimetre through the soil in a day. The diffusion coefficient for phosphate ion in water is 0.89×10^{-5} cm^2s^{-1}. Phosphorus diffusion through soil is slower than in pure water for three reasons: (i) soil water occupies only part of the soil so the cross-sectional area for diffusion is less; (ii) the diffusion path is tortuous because the water is present as films around soil particle; and (iii) most of the diffusable phosphorus is adsorbed on soil surfaces which equilibrates with and buffers the small amount of phosphorus in soil solution.

According to Sibbesen (1983) the P uptake of a plant root over a period of time depends on: the initial concentration of P in soil solution; the soil medium for P-diffusion; and the P dissolution of solid phase P, as a function of decreasing solution P-concentration time changing activity of HCO_3^- and H^+ in the rhizosphere, changing activity of exuded organic anions and changing activity of phosphate precipitating cations.

All the factors that govern the rate of phosphorus diffusion to the root and the extent of root growth are important in determining the availability of phosphorus to growing plants in a soil.

1.4. Adsorption and release of phosphate in soil

The immediate source of phosphate-P to plant roots is the soil solution. Phosphate deficiency in soil usually occurs from too low concentration of orthophosphate in the soil solution, rather than from an inadequate total P content. The concentration of P in the solution is governed by a dynamic equilibrium between solid and solution phases, in which phosphate is continually released from and re-adsorbed by the solid phase. Any change in the P concentration of soil solution will initiate physico-chemical processes to re-establish the equilibrium. These processes play a major role in the availability of phosphorus to plants as well as in improving the efficiency in fertilizer use. A decrease in the concentration of P due to plant uptake, tends to dissolve and/or desorb P from solid phase. The opposite processes, P precipitation or adsorption will come into effect, when soluble phosphate fertilizer is added to the soil. Thus the concentration of phosphate in the soil solution at any time is governed by both by the root uptake and adsorption or release of phosphate by solid phase (Khasawneh, 1971).

1.4.1 Adsorption of phosphate in soil

Phosphate adsorption is a process in which phosphate ions in solution react with atoms on the surface of soil particles. The extent to which it occurs is an important property affecting both the availability of phosphate to plants and the effectiveness of phosphatic fertilizers. When soluble phosphate compounds are added to the soil thus undergo a series of complex reactions. These compounds react rapidly with soil minerals, by precipitation reactions and adsorption onto surfaces, and the availability of this added P declines. In soils the problem of differentiating the adsorption from precipitation is made difficult by the fact that new bulk solid phases can precipitate onto the surfaces of existing solid phases and that weathering solids may provide host surfaces for the more stable phases into which they transform. The chemistry of these reactions between solids and solution is of fundamental importance in agriculture.

Soil particle surfaces, whether they be of the constant or variable charge type, are able to hold ions derived from the soil solution in close proximity to the solid. This acts as a potential store of nutrients able to replenish the soil solution when they are removed by the roots or microbial uptake. To study the phenomenon at the surface, subsamples of a soil are equilibrated at constant temperature with a number of solutions

containing different concentrations of phosphate and then the amount remaining in solution after adsorption is determined. The time of equilibrium varies with the system being studied. A simple cation exchange may be essentially completed within minutes, whereas the adsorption of orthophosphate can continue increasing for two days and even after this proceed at a very slow rate for some months (Wild, 1988).

The equilibrium between solid phase and solution phase P is usually expressed by the buffering capacity of a soil in the shape of adsorption isotherm, which is a line showing the relationship between quantity of P adsorbed by the soil and the changing concentration of P in the surrounding solution. According to Holford (1989) the buffering capacity or sorptivity of a soil is controlled by the two fundamental soil properties: one is the extent or number of P-reactive sites, and the other is the affinity of these sites for P. These processes may be on the soil colloidal surfaces (adsorption) or in the solution (precipitation). Numerical estimates of these properties can be obtained by fitting a suitable equation such as Langmuir or Freundlich equation to the adsorption isotherm. Some parameters of adsorption can be obtained from these equation to compare the behaviour of P in soils.

1.4.1.1 Mechanism relevant to surface reaction of phosphate

Phosphate ions are negatively charged and they have a high chemical affinity for surfaces in soils (Barrow, 1989). The chemical affinity for the surface is an important property and it differs amongst the phosphate ions in solution. Phosphoric acid has three H atoms and it loses these atoms gradually as the pH increases in the following order:

$$H_3PO_4 \quad <===> \quad H^+ \quad + \quad H_2PO_4^- \qquad pK1 = 2.148$$

$$H_2PO_4^- \quad <===> \quad H^+ \quad + \quad HPO_4^{2-} \qquad pK2 = 7.198$$

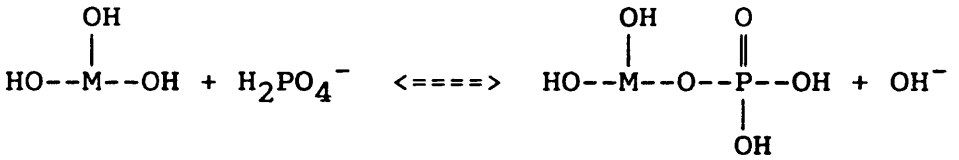
$$HPO_4^{2-} \quad <===> \quad H^+ \quad + \quad PO_4^{3-} \qquad pK3 = 12.375$$

The surfaces on which phosphate ions are adsorbed may be of two types: (a) surfaces of constant charge, i.e. the crystalline clay minerals which interact with phosphorus principally through the cations held tightly to their plate-like electrical surfaces; and (b) surfaces of variable charge, including the Fe(III) and Al oxides and organic matter, for which H^+ and OH^- determine the surface charge and potential, and calcite for which Ca^{2+} and CO_3^{2-} are the potential-determining ions.

In addition to these two distinctive groups there are some clay minerals such as kaolinite, which have pH-dependent charges on their crystal edges. Amorphous clay minerals such as allophane has also pH-dependent charge.

These react with phosphorus through their cations held by coulombic forces.

Hydrated Fe and Al oxides provide the most important surfaces of variable charge except in peat and highly calcareous soils. The charge on hydrous oxide structures is dependent on the pH and electrolyte content in the surrounding medium. These oxides are the most reactive cationic centres at low pH (Lafleur and Craddock, 1967). They proposed a general soil/phosphate reaction for the acid soils:



Where M is Al or Fe

They further reported that native phosphate and added water-soluble phosphate are "immobilized" to a higher degree by acid soils. Addition of high-affinity anions (e.g., citrate and humate ions) which compete with phosphate anions for cationic soil sites reduces the amount of phosphate immobilized and increases the amount of phosphate in the soil solution

Many hydrous oxide surfaces generate a significant number of positive charges as the pH falls. Goethite (α-FeOOH) for example, is positively charged below a pH of 8-8.5, its point zero of charge (p.z.c.) i.e. the positive and negative charges on the surface are balanced

(Mott, 1981). Oxides differ in their p.z.c.; for gibbsite (τ-$Al(OH)_3$) it is close to 9 whilst for silica it is close to pH 2. Depending on the crystal structure, the hydroxyls are one, two or three-coordinated to the metal (M) atoms. The O-H bond in two and three-coordinated hydroxyls is strongly polarized and hence acidic; singly coordinated hydroxyls are more basic and are potentially exchangeable by anions in solution (White, 1980). The goethite surface at the pH of p.z.c. should have an equal numbers of opposite charges, but to maintain this state the solution has to contain OH^- in a roughly hundred times excess over H^+ (Mott, 1981). This means that the goethite surface holds on to protons more strongly than water does. Silica on the other hand holds them less strongly, since even at pH 3 the surface is negatively charged.

At a pH lower than the p.z.c., anions in solution are attracted to the positively charged oxide surface to form an electrical double layer. Specific forces enhance the adsorption of phosphate over the indifferent anions like ClO_4^-, Cl^- and NO_3^-.

Specifically adsorbed ions have strong affinity for a clean hydroxylated surface and displace the existing ligand to cation (Mott, 1981).

$$\text{Oxide-M-OH}_2\Big]^{x+} + H_2PO_4^- \text{--->Oxide-M-O-PO}_3H_2\Big]^{(1-x)-} + H_2O$$

$$\text{Oxide-M-OH}\Big]^{(1-x)-} + H_2PO_4^- \text{--->Oxide-M-O-PO}_3H\Big]^{(1-x)-} + H_2O$$

The reactions which are thought to take place at the surface during the adsorption of phosphate are given by Goldberg and Sposito (1984) as follow:

$$SOH + H_3PO_4 \quad <====> \quad SH_2PO_4 + H_2O$$

$$SOH + H_3PO_4 \quad <====> \quad SHPO_4^- + H_2O + H^+$$

$$SOH + H_3PO_4 \quad <====> \quad SPO_4^{2-} + H_2O + 2H^+$$

Where groups prefixed by S represent surface sites. The three species proposed in this case may be regarded as dissociating so that, at low pH the uncharged SH_2PO_4 dominates and as the pH increases, the singly charged $SHPO_4^-$ and then doubly charged SPO_4^{2-} are found.

1.4.1.2 Factors influencing adsorption of phosphate

1. <u>Soil reactive sites</u>: The adsorption reactions are affected by the type and nature of the reactive sites contacted by phosphate in the soil solution.

The oxides and hydrous oxides of iron and aluminium have the capacity to adsorb very large amount of phosphorus. These occur as discrete particles in soils or as coatings or films on other soil particles. They also exist as amorphous aluminium hydroxy compounds between the layers of expandable aluminium silicates. Soils with significant contents of iron and aluminium oxides, have large phosphorus fixation capacities because of their high surface areas. Tisdale et al. (1985) suggested that

crystalline hydrous metal oxides are usually capable of retaining more phosphorus than layer silicates.

Phosphorus is adsorbed to a greater extent by 1:1 than 2:1 types of clay. Soils high in kaolinite, such as those found in areas of high rainfall and high temperature, will retain larger quantities of added phosphate than those containing the 2:1 types. Greater adsorption of phosphate in the former case is probably due to the higher amounts of hydrated oxides of iron and aluminium associated with the kaolinitic clays. In addition, kaolinite develops pH-dependent charges on its edges which can enter into adsorption reactions with phosphate. Clays such as kaolinite with a low SiO_2/R_2O_3 ratio will adsorb larger quantity of phosphorus than the clays with a high ratio. A large number of exposed hydroxyl groups in the gibbsite layer of kaolinite are exchangeable with phosphate and cause more adsorption.

The clay content of a soil has great impact on phosphate adsorption. Soils containing large quantities of clay will adsorb more phosphate than those with less clay content. In other words, the more surface area exposed with a given type of clay, the greater the tendency to retain phosphate.

Phosphate retention is closely related to the amorphous colloids content of a young developed soil. Volcanic ash is an important parent material of such soil and its weathering imparts a high phosphorus adsorption

capacity to the soil. Amorphous aluminosilicate minerals like allophane have a large negative charge which is partly or entirely balanced by complex aluminium cations (Imai et al., 1981; Tisdale et al., 1985). Phosphorus becomes adsorbed by reacting with these aluminium cations.

The amount and reactivity of calcium carbonates influence phosphate adsorption in soils. Impure calcites and those of high specific surface result in more adsorption of phosphate and more rapid formation of calcium phosphate precipitates. The activity of phosphorus will be lower in those soils that have a high Ca^{2+} activity, a large amount of highly reactive calcium carbonate, or a large amount of calcium-saturated clay. In order to maintain a given level of phosphate activity in the soil solution, it will be necessary to add larger quantities of phosphate fertilizers to such soils (Tisdale et al., 1985).

2. **The effect of soil pH**: Soil pH has profound effect on the amount and manner in which soluble phosphate become adsorbed. Adsorption of phosphorus by iron and aluminium oxides declines with increasing pH (White, 1980). Gibbsite [τ-$Al(OH)_3$] adsorbs the greatest amount of phosphate between pH 4 and 5. Phosphorus adsorption by goethite
(α-FeOOH) decreases steadily between pH 3 and 12 (Huang, 1975). Phosphate availability in most of the soils is at

a maximum in the pH range 6.0 to 6.5 (Tisdale et al., 1985). At lower pH values the retention results from the reaction with iron and aluminium and their hydrous oxides. Above pH 7.0 the ions of calcium and magnesium and their carbonates cause precipitation of added phosphorus, which decreases its availability. Lajtha and Bloomer (1988) regarded, calcium carbonate as the primary geochemical agent capable of retaining P in the soils of a desert ecosystem. Tiessen et al. (1984) suggested that Ca-bound forms of P constitute the dominant pool of mineral soil P in arid and semi-arid soils of the southwestern United States. The presence of calcium or magnesium ions must accompany high pH values. If the increase of these ions continued, the decrease in solubility of soil phosphorus. However, liming acid soils increases the solubility of phosphorus.

3. **Effect of anions**: Both organic and inorganic anions compete with phosphate for adsorption sites to varying extents. In some cases it may result in a decrease in the adsorption of added phosphate or a desorption of retained phosphate. Weakly held inorganic anions such as nitrate and chloride are of little significance, whereas specifically adsorbed anions like hydroxyl, sulphate and molybdate are competitive. The strength of bonding of the anions with the adsorption surface determines the competitive ability of that anion. For example, sulphate, even though considered to be specifically adsorbed anion, is unable to desorb much phosphate (Zhang et al., 1987).

Organic anions from various sources can affect the phosphate adsorption and desorption reactions in soils. The impact of organic anions on reduction of phosphate adsorption is related to their molecular structure and the pH of the system. Oxalate and citrate are specifically adsorbed on soil surfaces in a similar way to phosphate. The anions that are most effective in replacing phosphates are citrate, oxalate, tartrate, malate, and malonate. Some of these effects of organic anions on phosphorus retention are beneficial for its availability.

4. **Effect of organic matter**: Several authors noted correlation between organic C and the amount of P adsorbed by soils (Woodruff and Kamprath, 1965; Ahenkorah, 1968). According to Tisdale et al. (1985) the availability of phosphorus increased from decomposition of organic residues has been due to: (a) the formation of phosphohumic complexes which are more easily assimilated by plants, (b) anion replacement of the phosphate by the humate ions, and (c) the coating of sesquioxide particles by humus to form a protective cover and thus reduce the phosphate retention capacity of the soil. It was suggested that certain organic anions form stable complexes with iron and aluminium, thus preventing their reaction with phosphorus by blocking the adsorption sites (Leaver and Russell, 1957). It was further stated that these complex ions release phosphate previously retained by the same mechanism. Harter (1969) suggested that it is

OH groups in organic matter which effect phosphate adsorption through anion exchange, while the results of Appelt et al. (1975) showed that it is the Al and to lesser extent the Fe adsorbed by the organic colloids which are active in P adsorption. Organic matter does lower the adsorption of P, it also provides a method of increasing the P availability without the use of fertilizers. The evolution of carbon dioxide after the decomposition of organic residues has a favourable effect on phosphate availability. This gas is dissolved in water to form carbonic acid, which is capable of decomposing certain primary soil minerals.

On the basis of available evidence, it is clear that the addition of organic materials to mineral soils may increase the availability of soil phosphate.

5. **Effect of temperature**: Temperature affects most physical processes and the speed of chemical reactions generally increases with a rise in temperature. Barrow (1979) has described three effects of temperature on phosphate in soil, which are not always distinguished: the position of the equilibrium between phosphate in solution and adsorbed phosphate, the rate of transfer from adsorbed to firmly-held, and the rate of transfer from firmly-held to adsorbed. If the temperature at which phosphate reacts with soil is increased, the rate of reaction considerably increased (Barrow, 1989). It has also an important theoretical application. As the

temperature increases, the kinetic energy of the molecules increases. This increase is the increase in activation energy, which enables them to jump over the energy barriers into the new reaction state.

High temperatures are expected to slightly increase the molar solubility of compounds such as apatite, hydroxyapatite, octacalcium phosphate, variscite and strengite. Increase in temperature also stimulates the biological activity and the phosphate is released from organic residues. The dissolution of water-soluble phosphorus and resultant reactions with soil components to produce less soluble reaction products are hastened by higher temperatures. There was 33% reduction in concentration of water-soluble phosphorus for each 15^{o} C increase in temperature (Tisdale et al., 1985). The soils of the warm regions of the world generally adsorb more phosphates than the soils of temperate regions. These warmer climate also give rise to soils with higher contents of the hydrous oxides of iron and aluminium. Many workers are agreed that phosphorus retention increases at higher temperatures (Muljadi et al., 1966; Kuo and Lotse, 1974; White and Taylor, 1977).

6. **Effect of time**: Phosphorus adsorption by soils and many soil components follows two distinct pattern: an initial rapid reaction followed by a very much slower reaction. The adsorption reaction involving exchange of phosphate for anions and ligands on the surface of iron

and aluminium oxides are extremely rapid (White, 1980; Tisdale et al., 1985). The slower continuing adsorption reactions may involve such changes as (a) diffusive penetration or chemisorption of surface-adsorbed phosphorus into soil constituents, e.g., the incorporation of phosphorus into hydroxylaluminium or iron polymers and the occlusion of phosphorus in the surface of calcite as suggested by Griffin and Jurinak (1973), and (b) the precipitation of a phosphorus compound for which the solubility product has been exceeded (Veith and Sposito, 1977). These slow reactions involve a shift in the form of phosphorus held at the surface from more loosely bound to more tightly bound types which are less accessible to plants. An important practical aspect of time is the period after application of fertilizer. On some soils with high adsorption capacity the period of reaction is short, whereas on others the period may last for months or even years. The reaction of phosphate with soils can probably never be considered entirely complete as long as an appreciable concentration of phosphate remains in solution.

7. **Solid : solution ratio**: The relationship between adsorbed phosphate and solution concentration is influenced by solid : solution ratio. Narrow ratios of solid : solution (1 : 10 to 1 : 2) have been used experimentally and are considered to be closely related to the field conditions (White, 1980). Muljadi et al. (1966) used wider ratios (1 : 5000 and 1 : 10000) in

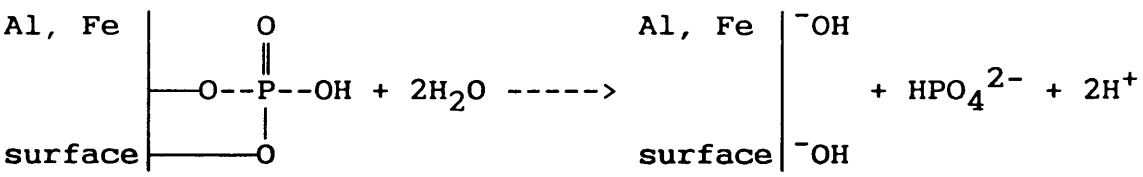
studying P adsorption by kaolinite and Al_2O_3 so that the change in solution pH on adsorption was minimized, but Rajan et al. (1974) and Helyar et al. (1976) achieved the same results at narrow solid : solution ratios. Soils recently fertilized or depleted of P by plants give Q : I plots for which the slope and equilibrium P concentration (EPC) vary with soil : solution ratio (White, 1966). This variation was eliminated by prolonged incubation at constant temperature (White and Taylor, 1977). Hope and Syers (1976) achieved the same results when phosphate solution was mixed with the soils was proportional to the reciprocal of time i.e., for infinite time. They concluded that at equilibrium the effect of solution : soil ratio disappeared.

1.4.2 Release of phosphate in soils:

Soil is a complex multicomponent system in which H^+ or OH^- transfer between the solution and surfaces of variable charge can occur due to the buffering capacity of the clay surfaces, exchangeable Al^{3+} and organic matter. There is interest in knowing if phosphate held firmly in nonlabile forms can become available to plants. Desorption processes that characterize the release of adsorbed phosphate have been shown to be fast and slow (Wild, 1988). Fast desorption, which occurs by ligand exchange, appears to be complete in a few hours if the soil is finely ground and a sink, such as anion exchange resin, is provided for the desorbed phosphate. The rate

of the slow reaction may depend on slightly soluble calcium phosphates being dissolved, on low diffusion rates through porous minerals, or a desorption requiring higher activation energy. The rate of desorption decreases with time according to the function $Kt^{0.3}$ (Tisdale et al., 1985), where t is the period of desorption and K is constant. Increasing the period of contact between soil and phosphorus reduces the value of K. It is further stated that the desorption became very slow after about two days. The extent of desorption is dependent on the nature of the adsorption complex at the surface of hydrous iron and aluminium oxides. Bache and Ireland (1980) described the desorption of phosphate due to changes in H_3O^+ and Ca^{2+} concentrations in solution. They have given the following mechanisms for phosphate desorption:

(i) exchange of hydroxyl for phosphate chemisorbed on the soil surfaces:



(ii) dissolution of calcium phosphate phases:

e.g., $Ca_{10}(PO_4)_6(OH)_2 + 6H_2O \rightarrow 4Ca(OH)_2 + 6Ca_{2+} + 6HPO_4^{2-}$

Hydrolysis of P in solution according to:

$$HPO_4^{2-} + H_3O^+ \text{ <=====> } H_2PO_4^- + H_2O$$

must also occur, depending on pH. Desorption of phosphate in the vicinity of plant roots may however, be more influenced by local pH changes than by microbial production of organic anions (Tinker, 1980). The effect of a change in pH on phosphate desorption depends on the soil properties.

Various techniques have been used to measure the soil P desorption curve such as increasing the dilution of the soil or extracting with an anion exchange resin (Brewster et al., 1975) but the results does not often agree with the desorption curve calculated from plant P uptake. For example Kepert et al. (1979) found that the 'plant desorption' curve gave higher P concentration and had a steeper slope than the Q : I plot obtained by desorption into 0.01 M $CaCl_2$. However, anion exchange resin, analogus to plant roots (Amer et al., 1955), was evaluated to be the best method corresponding to P uptake by plants (Sibbesen, 1983).

1.5 Phosphorus requirements of plants

Phosphorus occurs in most plants in concentrations between 0.1 and 0.4 % (Tisdale et al., 1985), a range considerably lower than found for nitrogen and potassium. However, it plays an important role in seed formation, cell division, flowering and fruiting, root development,

strengthening straw, quality improvement and resistance to diseases. Of the many essential functions that phosphorus has in plant life, its role in energy storage and transfer is the most important. Phosphate compounds act as "energy currency" within plants. The most common phosphorus energy currency is adenosine di- and triphosphate (ADP and ATP). In addition to this vital metabolic role, phosphorus is an important structural component of a wide variety of biochemicals, including nucleic acids, coenzymes, nucleotides, phosphoproteins, phospholipids, and sugar phosphates. Being a component of DNA and RNA phosphorus contributes to developmental and hereditary processes in plants.

Plants can absorb phosphorus as either the primary $H_2PO_4^-$ ion or smaller amounts of the secondary HPO_4^{2-} orthophosphate ion. Since the former is most abundant over the range in soil pH prevailing for most crops, it is usually the principal form absorbed. Tisdale et al. (1985) reported that studies with some plants have shown as there are about 10 times as many absorption sites on plant roots for $H_2PO_4^-$ as there are for HPO_4^{2-}. Absorption of the $H_2PO_4^-$ species is greatest at low pH values, whereas uptake of the HPO_4^{2-} ion is the greatest at higher value of soil pH. The rate of P uptake also depends on the microbial environment, presence of other ions, phosphorus concentration near roots, soil temperature, soil texture and phosphorus movement, mostly by diffusion. Mass flow provides very little phosphorus

due to the low soil solution concentrations. Phosphate uptake is more dependent on aspect of plant root activity than is the case for other major nutrients.

One of the main factors influencing phosphorus uptake is the extent of root system in the enriched top soil. The more highly branched the root system, the greater the volume of soil that will be within diffusion range . Root hairs have been shown to be particularly important in extending the volume of soil explored and increasing the amount of phosphorus taken up. Many rhizosphere organisms compete with the root for phosphate where the phosphate concentration is low. Some microorganisms solubilize phosphate compounds of low solubility, by excreting acids. The importance of root association with particular microorganisms known as endotropic mycorrhyzae has been shown to increase phosphorus uptake by a number of crops. A mycorrhiza, known to be, vesicular-arbuscular mycorrhyza (VAM) has beneficial effect on P uptake (Fitter, 1992). It offers a solution for the acquisition of phosphorus.

Plants appear to have severe problems in getting phosphate at a very early stage in their development. So P deficiency symptoms most often occur in seedlings and young plants. Phosphorus is mobile within the plants, it is translocated from the older, first formed tissue to the growing points. This causes the deficiency symptoms on the lower leaves. Deficiency of phosphorus generally

causes stunted growth, dark green colour, associated with a purplish colour in the seedling stage. Inadequate supply of phosphorus generally causes delay in crop maturity and seed formation.

For efficient use of phosphorus, fertilizer requirements should be assessed as accurately as possible. Recommendations for phosphate fertilization must take into account the specific plant requirement, the available level of phosphate in the soil and the soil P adsorption characteristics. Bache and Williams (1971) have proposed that phosphate requirements of a soil can be estimated using quantity : intensity plots. Fox (1981/82) advocated that phosphate adsorption curves are the only method for precise fertilizer recommendations, and especially if the properties of the soil are unknown.

The phosphorus concentration considered to be critical for plants varies widely. Genotypic differences in the rooting habit and activity, along with varied yield potential, are the main factors responsible for this variation. Potatoes require a very high soil phosphorus level for maximum yield, while grasses and cereals can take up enough phosphorus despite a low soil phosphorus level (Archer, 1988).

1.6 Aim of thesis

Though the phosphorus is taken up by the plants in smaller amounts than nitrogen and potassium, its role in

plant life can not be over-emphasized. It is common practice for farmers to include phosphorus in their annual fertilizer management programmes. The practice of continuous fertilization has resulted in establishment of high phosphate levels in many regions of intensive agriculture, which may cause an imbalance of soil nutrients as well as matters of concern for the general environment such as eutrophication of sewage wastes. On the other hand such residual phosphorus can be utilized by complementing fresh application.

Deficiency of phosphate is still common in many parts of the world. Under severe conditions the soil may not supply any phosphate at all to a crop. The subject of availability of phosphate to plants has remained the centre of research efforts for over a century, yet its behaviour is imperfectly understood. Because of small quantities of phosphorus in soils and its tendency to react with soil components to form relatively insoluble compounds, many of which have limited its availability to plants.

The aim of this project is to assess the phosphate status of different types of soil by various conventional procedures. The objective of this part of study will be to find relationships between various extractants and other soil properties. By comparing the efficiency of various P extractants, it is intended to select an appropriate extractant for different types of soils.

The relation between the concentration of phosphate in solution and that in solid phase accounts for the availability of this nutrient element to plants. So to investigate this relationship phosphate adsorption studies will be carried out. The retention of phosphate in acid soils is a severe problem in limiting its availability to plants. This retention behaviour of acidic soils after liming will also be studied.

The rapid replenishment of phosphate from solid phase to soil solution contributes more towards its availability. It depends mostly on the desorption of phosphate. So this part of study will cover the phosphate desorption behaviour of some soils. The availability of phosphate to plants will be investigated through pot experiment, in terms of yield and phosphate uptake.

CHAPTER 2

ROUTINE ANALYTICAL METHODS AND SOILS

2.1 Measurement of soil pH

The pH of the soil was measured electrometrically by a combined glass/reference electrode. The pH was measured in a standard 1:2.5 soil-water or 0.01 M $CaCl_2$ solution. The pH meter was first standardized with buffer solutions of pH 7.0 and 4.0, for neutral to acidic soils. While standardization with buffer pH 7.0 and 9.0 was done for alkaline soils. 8 g of air-dried soil was weighed into two sets of 30 cm^3 screw-capped glass bottles. In one set of samples, 20 cm^3 deionized water was added to each bottle with a 20 cm^3 dispenser. Similarly 20 cm^3 0.01 M $CaCl_2$ solution was added to each bottle in the second set of samples. The tightly capped bottles were shaken on an orbital shaker at 25^o C for half an hour. The electrode of standardized pH meter was immersed in the suspension, stirred by swirling slightly and the pH reading was recorded.

2.2 Percent moisture determination

Percent moisture was determined gravimetrically. 100 g soil was weighed in a clean weighed basin, was kept in oven at 110^o C for overnight. The basin was then cooled

in desiccator for half an hour and re-weighed. Percent moisture was calculated on oven-dried basis as follows:

$$\frac{\text{Weight of fresh soil - Weight of oven-dried soil}}{\text{Weight of oven-dried soil}} \times 100$$

2.3 Organic matter determination

2.3.1 Loss on ignition

Vitreosil basins were washed thoroughly and then placed in an oven at 110^{o} C for one hour. Then the basins were cooled in desiccator for 30 minutes and their weight was recorded separately for each sample. 5 g air-dried soil was weighed in each basin. The basins were placed in the oven overnight to remove the soil moisture. The basins were transferred to desiccator and cooled for half an hour. The basin were reweighed. The basins were then placed in electric furnace in an order. As the ink marks disappear during ignition, the order of basins position was recorded on a paper for identification. The furnace was switched on, the temperature was increased to 450^{o} C. After 6 hours the furnace was switched off. The basins were transferred to desiccator with the help of furnace tongs, for cooling. After half an hour the basins were reweighed. The organic matter content was calculated as a percent loss on ignition.

$$\text{Percent loss on ignition (\% LOI)} = \frac{\text{Weight of oven-dried soil - Weight of ignited soil}}{\text{Weight of oven-dried soil}} \times 100$$

2.3.2 Dichromate method for organic matter

This method has been recommended by MAFF (1986)

2.3.2.1 Reagents

(i) Barium diphenylamine sulphonate

0.5 g barium diphenylamine sulphonate was dissolved in approximately 250 cm^3 of warm deionized water. Complete dissolution was made by using a magnetic stirrer. 50 g of barium chloride was added and dissolved by warming. The solution was cooled and the volume was finally made up to 500 cm^3 with water.

(ii) Ferrous sulphate 0.4 M

5 cm^3 concentrated sulphuric acid was added to into 1500 cm^3 deionized water. 320 g of ammonium ferrous sulphate was dissolved in the acidified water and the volume was made up to 2 litres.

(iii) Potassium dichromate 66.7 mM

Approximately 80 g of crystalline potassium dichromate was dried in an oven at 110^{o} C overnight and then cooled in a desiccator. 39.24 g of the dried salt was dissolved in approximately 700 cm^3 water. 800 cm^3 of concentrated sulphuric acid was added carefully in fumecupboard and cooled. 400 cm^3 of orthophosphoric acid was added and stirred to dissolve the chromic acid. Finally the volume was made to 2 litres.

(iv) Standardization of ferrous sulphate solution

40 cm^3 of potassium dichromate solution was taken with 20 cm^3 pipette in a 500 cm^3 flat bottomed (quick fit) flask, 100 cm^3 of water was added and 2 cm^3 of barium diphenylamine sulphonate was added as indicator. The contents in flask were titrated against 0.4 M ferrous sulphate solution. Ferrous sulphate solution was added dropwise from a 50 cm^3 graduated burette until the colour of the contents of flask changed from purple to bright green. The volume of ferrous sulphate used was noted. The standardization factor was calculated from the average of three readings.

Standardization factor of ferrous sulphate

= 40/volume of ferrous sulphate used.

The standardization of ferrous sulphate was carried out each day immediately before use.

2.3.2.2 Procedure

0.25 to 1.0 g (upper limit for soils containing low organic matter) air dried and finely ground soil sample was accurately weighed into 500 cm^3 flat bottomed quickfit flask. 40 cm^3 of potassium dichromate solution was added. The air condensers were fitted to the flasks. The flasks were placed on a sandbath with controllable temperature of approximately 130^{o} C for two hours. After heating, the flasks were removed from the sandbath and

cooled. 100 cm^3 of water and 2 cm^3 of barium diphenylamine sulphonate were added. The samples were titrated with standardized ferrous sulphate reagent solution by adding dropwise until the colour changed from purple to bright green. The volume of the ferrous sulphate used was noted. A blank titration was carried out exactly in the same way except that no soil sample was added. The calculation was carried out as follows:

% Total organic carbon =

$$\frac{\text{(Blank titre - Sample titre) x 3 x 100 x M}}{\text{(Weight of oven-dried soil) x 1000}}$$

Where M is the concentration of the ferrous sulphate solution used after standardization.

2.4 Textural class determination of soils

This method was modified by Khan (1987) from the method used by MAFF (1986)

2.4.1 Reagents

(i) 30 % hydrogen peroxide

(ii) Dispersing reagent (Calgon)

50.0 g sodium hexametaphosphate plus 7.0 g sodium carbonate (anhydrous) were dissolved in water and diluted to 1 litre.

(iii) Silicon antifoaming agent

1 cm^3 of 30% aqueous emulsion (BDH Chemicals Ltd.) was diluted to 100 cm^3 in water

(iv) 2 M hydrochloric acid

2.4.2 Procedure

2.4.2.1 Dispersion

10 g of air dried soil passed through 2 mm sieve was weighed, from each sample, accurately into 600 cm^3 beakers. Approximately 10 cm^3 of 30% hydrogen peroxide and 2 drops of antifoaming agent were then added to each beaker. The initial reaction was allowed to subside for 20 minutes. The beakers were then gently heated on a steam bath with occasional stirring with a glass rod. The heating was continued until the reaction ceased. The beakers were cooled, a further 10 cm^3 of 30% hydrogen peroxide was added washing down the sides of the beaker and heating continued until the reaction ceased completely. 20 cm^3 of hydrogen peroxide was sufficient for most of the soils except those high in organic matter where addition were continued until no reaction was observed.

For soils containing calcium carbonate, approximately 2 M hydrochloric acid was added dropwise and the contents of the beaker were stirred continuously until the effervescence ceased. For all soils a further

10 cm^3 of the acid was added for a dispersion of soil. The beakers were stirred at intervals during an hour and then the soil was allowed to settle. The soil suspension was filtered through a Whatman filter paper No. 50 under suction. The filter paper was washed with three successive portions of 50 cm^3 of hot water. The soil was scraped from the filter paper with a spatula and then the filter paper and spatula washed with a jet of hot water letting the washings into the beaker. The sides of the beakers were washed down with distilled water and sufficient water was added to give approximately 2 cm depth of suspension in the beakers. 10 cm^3 of Calgon solution was pipetted into each beaker. The suspension was dispersed for 5 minutes using the ultrasonic probe (MSE Instrument Ltd.). After the dispersion, any soil adhering to the probe was washed into the beaker using distilled water.

2.4.2.2 Fractionation of sand

A one litre graduated cylinder was set up with a large filter funnel in the neck for each soil. A 180 µm mesh and a 53 µm mesh sieves were banked together and placed in the funnel with 180 µm mesh sieve on the top. These sieve sizes allow the separation of the coarse plus medium and fine sand fractions. The soil suspension was poured into the 180 µm mesh sieve. The sides of the beakers were washed using a wash bottle and a rubber coated glass rod to ensure that all the soil was removed

from the beakers. The soil was washed through the 180 μm mesh sieve until the coarse and medium sand appeared clean. The contents of the 53 μm mesh sieve were washed in the same way. The sieves and funnel were removed and the volume of the cylinder was made up to 1000 cm^3 with distilled water. The contents of the both sieves were washed completely into two separately weighed and labelled porcelain basins and evaporated to dryness on a steam bath. The basins were then transferred to 110° C oven and left overnight, cooled in a desiccator for 30 minutes and reweighed. The percent coarse plus medium sand and fine sand was then calculated on the oven dry basis in each soil sample.

2.4.2.3 Fractionation of silt plus clay

After removing the sand fractions, the cylinders were kept at a constant room temperature of 20° C. The temperature of the suspension was noted and appropriate time for the silt plus clay at a depth of 20 cm and sampling depth of 10 cm for clay particles were selected from the table prepared by the Soil Survey of England and Wales (Hodgson, 1976). Each cylinder was shaken thoroughly for one minute to ensure that all the soil was in suspension. The cylinder was then placed on the pipetting stand. Immediately a clean dry 25 cm^3 pipette was lowered down into the cylinder with the tap closed, until it just failed to touch the surface of the liquid and the height on the scale was noted. About 20 seconds

before the required time, the pipette was lowered down gently to exactly 20 cm depth. The tap was opened at the appropriate time and sample of slightly more than 25 cm^3 of the suspension was taken. The tap was closed and the pipette was then removed from the cylinder and the volume adjusted to 25 cm^3. The contents of pipette was washed into the weighed basin. The shaking was then repeated to obtain a duplicate sample in the same way. The samples were evaporated to dryness on the steam bath and then dried at 110^o C in an oven overnight. The samples were then removed, cooled in a desiccator for 30 minutes and weighed.

The cylinders were left undisturbed at a room temperature on a flat table for the appropriate time to allow the silt particles to settle down. The temperature of the suspension was noted and a sample of clay was taken at 10 cm depth by similar procedure as described for silt plus clay except that the cylinder was not shaken between pipetting the samples. Exactly 25 cm^3 content of the pipette was run into the weighed and labelled basin, evaporated to dryness on a steam bath and then left in a 110^o C oven overnight.

14.25 mg as the weight of calgon used in 25 cm^3 was subtracted from the weights of silt plus clay and clay.

Textural class of each soil was determined with the help of a triangular chart prepared by the Soil Survey of England and Wales (Hodgson, 1976)

2.5 Extractable iron and aluminium in soil

Amorphous oxides of iron and aluminium were extracted with acid ammonium oxalate (Tamm's reagent).

2.5.1 Reagents

(i) Ammonium oxalate $(CO_2NH_4)_2.H_2O$

(ii) Oxalic acid $(CO_2H)_2.2H_2O$

(iii) Extracting solution: 24.9 g Analar ammonium oxalate and 12.6 g Analar oxalic acid were dissolved in approximately 800 cm^3 deionized water and the volume was then made to 1 litre. This gave a solution of 0.175 M ammonium oxalate and 0.1 M oxalic acid at pH 3.3.

(iv) Working standard solutions: 1, 2, 3, 4 and 5 µg cm^{-3} of iron and aluminium standard solutions were made from 1000 µg cm^{-3} of iron and aluminium (BDH company prepared) solutions respectively. These working standard solutions were made by diluting 0.1, 0.2, 0.3, 0.4 and 0.5 cm^3 from 1000 µg cm^{-3} solution of each element in 100 cm^3 volumetric flasks.

2.5.2 Procedure

Duplicate 5 g air dried soil < 2.0 mm samples were shaken with 50 cm^3 extracting solution on an end over end

shaker for 2 hours. After filtering the soil suspension through Whatman filter paper No. 2, the concentrations of iron and aluminium were measured through absorbence on atomic absorption spectrophotometer (Perkin Elmer model 370A). Blank determination was also carried out along with the samples.

2.6 Automated determination of phosphate

2.6.1 Technicon autoanalyzer

2.6.1.1 Introduction

Accurate and rapid methods for phosphorus determination in soils and plant material are vital for research and diagnostic purposes. The method was based on the reduction of the phospho-molybdate complex by ascorbic acid. This method is the most versatile and widely used for soil and plant phosphorus determination. Colwell (1965) used the manual method of Murphy and Riley (1962) for the automatic analysis of sodium bicarbonate (Olsen's reagent) extractable phosphorus. Ng (1970) used a modified ascorbic acid method for Bray No. 2 extracts, and a vanadomolybdate method for phosphorus in perchloric-sulphuric acid digests of the soils for total phosphorus. Blakemore et al (1987) described autoanalyzer method, as the most widely used in New Zealand, for determination of main fractions of phosphorus.

The automated method is labour conserving and can handle large number of samples on routine basis with higher degree of reproducibility. It is also relatively more precise as each sample is treated unbiased. The human error in mixing and other operation is reduced to a great extent. The automated procedure can also tolerate slight turbidity in the extract which is sometimes unavoidable, for example in adsorption experiments where very little soil is used. Lennox (1979) reported that the automatic determination of phosphate was an excellent procedure as regard to recovery and effectiveness, the detection limit for the automated step was 1 µg P per litre.

Any autoanalyzer system can be upgraded by adding microprocessor based controller and data handler. The sampling rate and sample to wash ratio may be set to any desired rate and ratio. As a data handler, the microprocessor can provide drift, gain and carry over corrections through peak height adjustments. The microprocessor also produce a printed record of the calculated and corrected final analytical results.

2.6.1.2 Manifold design

The Technicon AutoAnalyzer II was used in this study for analysis of phosphate concentration in various test solutions and Kjeldahl digests of plant material. The system mainly comprised of a sampler, a pump, a constant temperature water bath and a colorimeter equipped with

appropriate filter, phototube and a blue coded bulb. The manifold used for the phosphorus analysis was set up as shown in Fig. 2.1. Results of the samples were recorded with a single pen chart recorder. The system was connected to a BBC microcomputer which was used with a software designed to measure the peak heights and/or the calculation of results.

For getting greater reproducibility and normal functioning of Technicon AutoAnalyzer II, some measures have to be taken. There should be no plastic tubings in the manifold because once the ascorbic acid has been added, the blue colour is adsorbed by plastic at high phosphorus concentrations and released at low phosphorus concentrations, resulting in poor wash out between samples. Moreover, a slightly longer version of the glass connecting tube inside the colorimeter is needed, to make the shortest possible connection of tubing to the flow cell. Silicon rubber tubing is used. There must not be stainless steel in the sample line, as it adsorbs and releases phosphorus depending upon its age. The sampler probe should be replaced with a glass capillary.

The reagents have to be relatively concentrated to remain stable, so to obtain the required total flow rate and final reagent concentrations, extra water is required if the sample flow rate is not to be large. The water conveniently contains the wetting agent and allows a degree of flexibility at low phosphorus samples, without

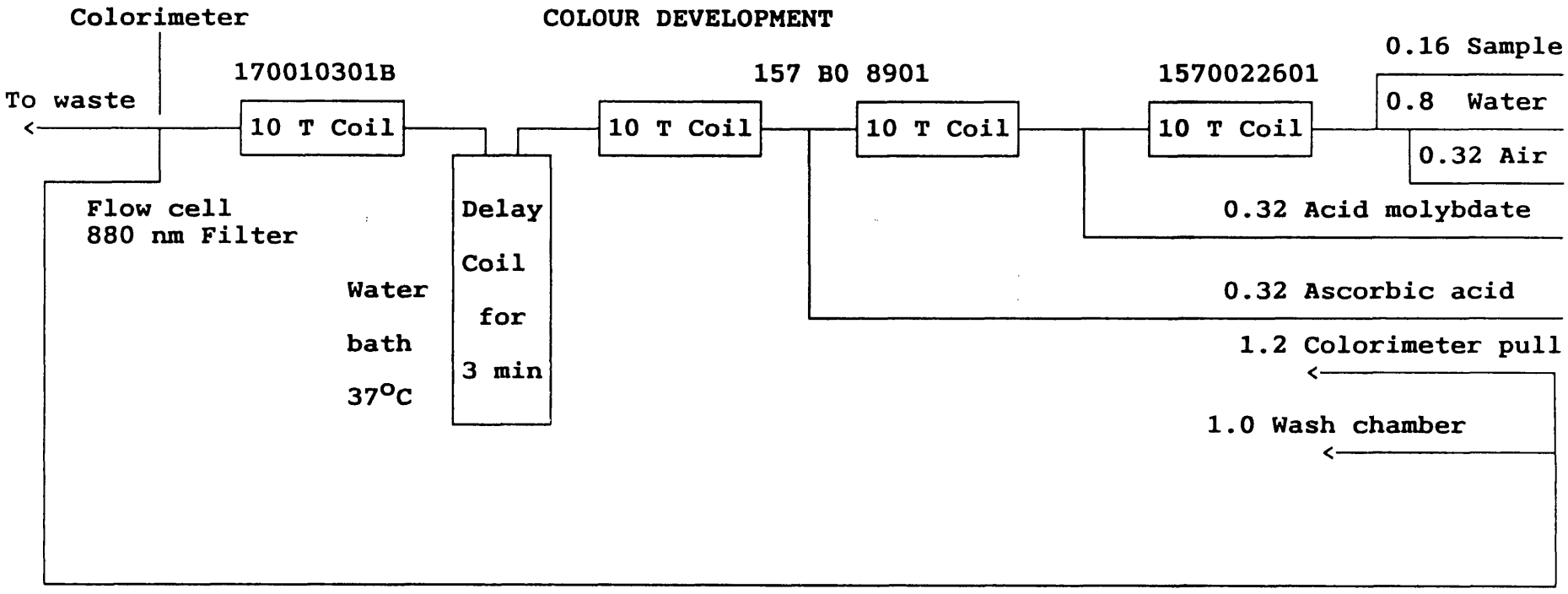


Figure 2.1 Technicon AutoAnalyzer II manifold for phosphate-P determination

the need for high scale expansions. The sample and water flow rates can be changed to suit different phosphorus ranges provided the total flow rate is approximately 1 cm^3 per minute. The system gives a linear response to phosphorus up to a maximum of concentration of approximately 0.8 µg P cm^{-3} in the reagent stream after addition of all reagents. The aim is to have a maximum of 0.5 µg P cm^{-3} in the reagent stream by selecting appropriate flow rates for the sample and water lines (Table 2.1).

Table 2.1 Appropriate flow rates for the sample and water lines in the system

----------Flow rate cm^3 per minute----------				Maximum phosphorus
Sample flow	Water flow	Sample + Water	Total Flow	in sample (µg cm^{-3})
0.16	0.80	0.96	1.60	5
0.42	0.60	1.02	1.66	2
0.60	0.42	1.02	1.66	1
0.80	0.23	1.03	1.67	1

With high sample flow rates there may be problem with the intersample air bubble. It is necessary to increase the concentration of wetting agent in the water as the water flow rate is decreased to maintain the final concentration. For P concentration > 5 µg cm^{-3} a dilution

step was incorporated into the manifold to reduce the P concentration to 0-5 μg P cm^{-3} range (Fig. 2.2).

2.6.2 Phosphate in soil extracts/solutions

The method is based on the formation of a phospho-molybdate complex, which is reduced by using ascorbic acid to produce a blue colour. The intensity of colour is measured at 660 or 880 nm. Lennox (1979) preferred 880 nm filter as its absorptivity is 50% greater than the 660 nm filter. Sarirullah (1989) also obtained better results with 880 nm filter. In order to speed up the formation of the complex, a small amount of antimony is also added. Tiong (1987) reported that the incorporation of potassium antimony tartrate to the method enhances colour production without heating to higher temperatures.

2.6.2.1 Reagents

(i) Acid ammonium molybdate

Ammonium molybdate, 5.2 g

Antimony potassium tartrate, 0.1 g

Concentrated sulphuric acid, 60 cm^3

The sulphuric acid was added carefully to approximately 800 cm^3 deionized water in the fume cupboard. After cooling, the ammonium molybdate was dissolved in the dilute acid. The antimony potassium tartrate was first dissolved in a small volume of

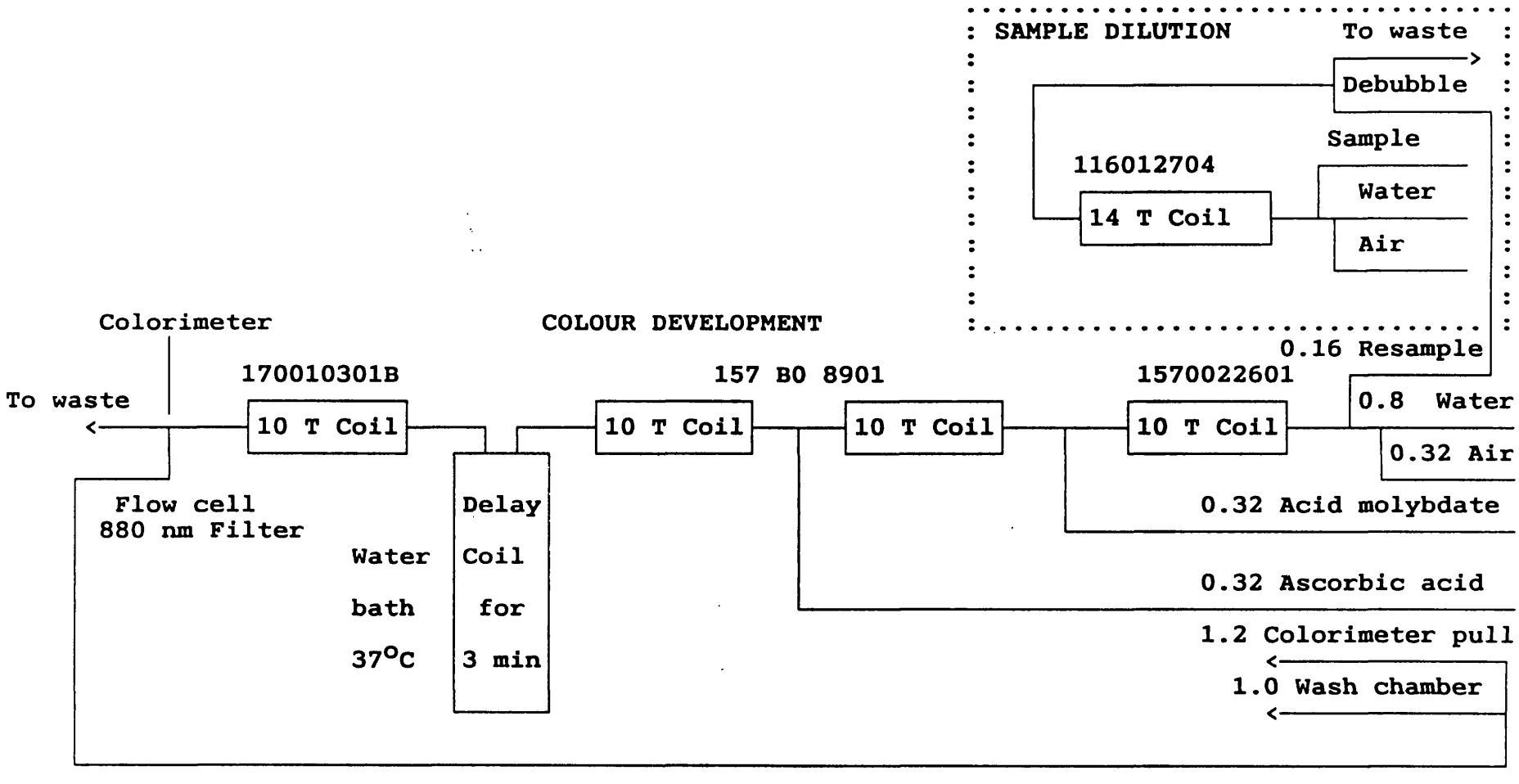


Figure 2.2 Technicon AutoAnalyzer II manifold for phosphate-P determination

deionized water, then added to the dilute acid by stirring to avoid precipitation. The volume was made up to 1 litre with deionized water. The solution was stored in a brown bottle away from light. The reagent was stable for one month.

(ii) Ascorbic acid: 0.75 g ascorbic acid was dissolved in 100 cm^3 deionized water. This solution was stable for one day.

(iii) Dilution water and Aerosol 22 (Wetting agent): 0.5 cm^3 Aerosol 22 was added to 1 litre deionized water

2.6.2.2 Procedure

Filtered soil extracts and other soil test solutions were analysed using the manifolds of Technicon AutoAnalyzer II, following the procedure of Sarirullah (1989).

The switches of colorimeter, water bath, chart recorder and computer were made on half an hour before running the actual experiment. The tubing system was set in according to the manifold (Fig. 2.1 or Fig. 2.2). The 880 nm filter and appropriate phototube were also inserted. The system was allowed to run on sampling deionized water for sometime, to establish an even flow and proper mixing of the reagents.

The small plastic cups were used in the sampler (rotary tray). Cups were carefully filled to the upper

mark, without touching the liquid to the fingers, to avoid contamination. A few cups of top standard and zero concentration were run in alternate position to set the range on the chart recorder. The samples cups were kept in following order: starting from 2 top standard cups, 1 wash cup, 2 zero cup, 10 sample cups, 2 top standard cups, 1 wash and 2 zero cups. The same sequence was followed throughout the experiment. The first set of sample cups was preceded by a pair of blank solution (zero concentration) cups. The samples in the cups were sucked through the capillary probe by the suction force of the pump. The response of colorimeter to the blue colour development was transmitted to the chart recorder in the form of a peak. The computer also recorded the same response, which was shown on the screen as well as in the printed form.

Sample and water flow rates were selected from the Table 2.1. The samples were normally run at the rate of 50 samples per hour. For concentrations above 5 μg P cm^{-3} the dilution system was introduced as in Fig 2.2, the rate was then reduced to 40 samples per hour.

There are a few points worth of consideration in using this procedure:

- Care should be taken that individual samples of high concentration among a set of predominantly low

concentration samples are not included in the same run.

- Brij-35 wetting agent used in many methods causes an intense blue colour, so no traces of the brij must enter the system e.g., from wash chamber where the brij is used for other methods.

- There is a slight tendency for the molybdate deposit to accumulate in the flow cell and transmission tubing, causing a slight drift in base line. A daily wash out of the manifold with 1 M NaOH solution is sufficient to remove this material. A wash out of the manifold with an acid wash followed by washing with deionized water is also important when the manifold has been used for alkaline extracts.

2.6.3. Phosphate in plant digest

Phosphate-P in acid digests containing selenium could not be determined using the ascorbic acid/molybdate blue method. However, phosphate was determined by the formation of a yellow phospho-vanado-molybdate complex absorbed at 420 nm. The method was followed according to the manifold given in Fig. 2.3. The determination was linear in the range of 0-10 µg P cm^{-3}.

2.6.3.1 Reagents:

(i) Ammonium molybdate/Ammonium metavanadate

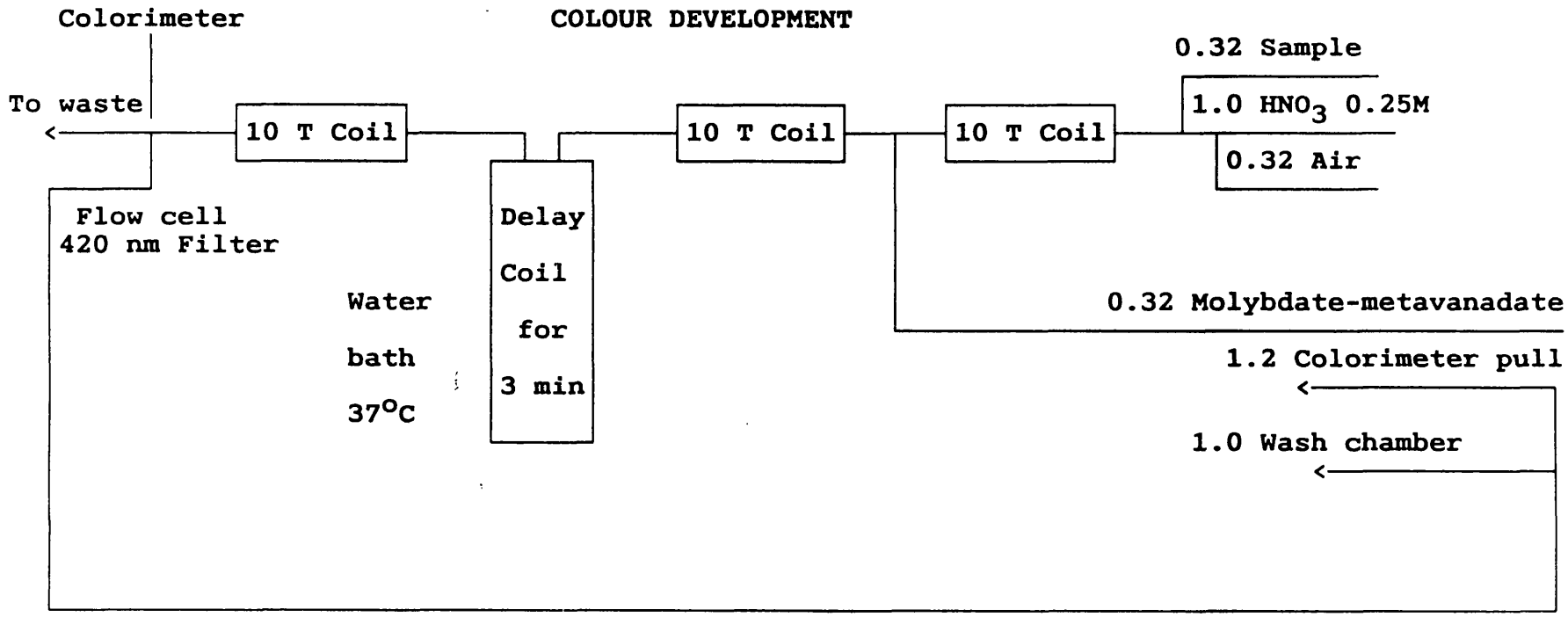


Figure 2.3 Technicon AutoAnalyzer II manifold for phosphate-P determination in plant material

Ammonium molybdate = 25 g

Ammonium metavanadate = 1.25 g

Levor IV = 1 cm^3

Final volume with deionized water = 1 litre

(ii) Nitric acid 0.8 M (0.25 M for acid digest)

(iii) Standard solutions of P in the range of 0-10 µg cm^{-3} were made up using a solution containing the same acid and catalyst concentrations as the samples and passed through the same process of digestion.

2.6.3.2 Procedure

The same procedure as described in Section 2.6.2.2 was adapted using different manifold (Fig. 2.3). The sampling rate of 50 per hour was used and the subsequent colour complex was determined at 420 nm filter with appropriate phototube.

2.7 Soil phosphate extraction methods:

2.7.1 0.5 M Acetic acid

2.7.1.1 Reagents

(i) 0.5 M acetic acid: 29 cm^3 of analar grade glacial acetic acid was added to approximately 800 cm^3 of deionized water in 1 litre volumetric flask, with

graduated cylinder. The volume was then made up, with deionized water to 1000 cm^3.

(ii) Phosphorus stock solution 1: 4.3937 g of oven dried KH_2PO_4 was weighed accurately, in a four digits electronic balance, in a beaker and dissolved with deionized water. The contents of the beaker was fully transferred to one litre volumetric flask and diluted up to the mark with deionized water. The solution strength was to be 1000 µg P cm^{-3}.

(iii) Phosphorus stock solution 2: 100 cm^3 of the stock solution 1 was diluted to one litre with 0.5 M acetic acid solution. This solution contained 100 µg P cm^{-3}.

(iv) Phosphorus working standards: 0.0, 1.0 and 5.0 cm^3 of phosphorus solution 2 were pipetted into 100 cm^3 volumetric flask and diluted to 100 cm^3 with 0.5 M acetic acid. The strength of this solutions was 0, 1 and 5 µg P cm^{-3} respectively.

2.7.1.2.Procedure:

2.5 g of air-dried soil sample, ground to pass a 2 mm sieve, was weighed into 4 oz screw cap glass bottle, in triplicate. 50 cm^3 of 0.5 M acetic acid was added to each bottle through a dispenser. The bottles were capped tightly and shaken in end over end shaker for 30 minutes. Three bottles of blank (without soil) were processed,

with each set of samples, in the same way. After shaking the contents of the bottles were filtered through Whatman filter paper No. 2 into 60 cm^3 polyethylene bottles. The phosphorus concentration was measured colorimetrically, using Technicon Autoanalyzer II by the method given in Section 2.6.2.2.

2.7.2. Sodium acetate buffer (Morgan's solution)

2.7.2.1 Reagents:

(i) 0.5 M Morgan's solution: 150 cm^3 glacial acetic acid was added to approximately 4000 cm^3 deionized water and 500 g sodium acetate was dissolved and the pH of the extracting solution was adjusted to 4.8. The volume was then made up to 5 litres with deionized water.

(iii) Stock standard solution of phosphorus: 0.4394 g dried KH_2PO_4 was weighed accurately and dissolved in deionized water. The solution was diluted to 1000 cm^3 with deionized water. This solution contained 100 µg P cm^{-3}.

(iv) Working phosphate standard solution: 1 cm^3 and 5 cm^3 of the stock phosphate standard solution was diluted with sodium acetate buffer solution. This solution contained 1.0 and 5.0 µg P cm^{-3}, respectively.

2.7.2.2 Procedure:

2.5 g of air-dried soil samples were shaken, with 50 cm^3 Morgan's solution in screw capped 4 oz bottles, in

the same way as in acetic acid extractant (Section 2.7.1.2). The contents of the bottles were filtered through Whatman No. 2 filter paper. The phosphorus concentration was measured by the method given in Section 2.6.2.2. Blank determination was also carried out with each batch of samples.

2.7.3 Ammonium acetate buffer (Modified Morgan's Solution)

2.7.3 1 Reagents:

(i) 0.5 M modified Morgan's solution: 150 cm^3 glacial acetic acid was added to approximately 4500 cm^3 deionized water and 192.5 g ammonium acetate was dissolved and the pH adjusted to 4.8, by adding 50% NaOH solution drop-wise. The volume was then made up to 5 litres with deionized water.

(ii) Stock standard solution of phosphorus: 0.4394 g dried KH_2PO_4 was weighed accurately and dissolved in deionized water. The solution was diluted to 1000 cm^3 with deionized water. This solution contained 100 µg P cm^{-3}.

(iii) Working phosphate standard solution: 1 cm^3 and 5 cm^3 of the stock phosphate standard solution was diluted with ammonium acetate buffer solution. This solution contained 1.0 and 5.0 µg P cm^{-3}, respectively.

2.7.3.2 Procedure:

2.5 g of air-dried soil samples were shaken, with 50 cm^3 modified Morgan's solution in screw capped 4 oz bottles, in the same way as in acetic acid extractant

(Section 2.7.1.2). The contents of the bottles were filtered through Whatman No. 2 filter paper. The phosphorus concentration was measured by the method given in Section 2.6.2.2. Blank determination was also carried out with each batch of samples.

2.7.4.Sodium bicarbonate extractable phosphorus

2.7.4.1 Reagents:

(i) 0.5 M sodium bicarbonate: 210 g sodium bicarbonate was dissolved in 4 litres of deionized water and 25 cm^3 polyacrylamide solution was added. The pH was adjusted to 8.5 with 50% sodium hydroxide at 20^oC. The volume was made up to 5 litres with deionized water. The solution was stored in a polyethylene container for use not longer than one month. The pH of solution was checked each time before use.

(ii) 4 N sulphuric acid: 110 cm^3 analar grade concentrated sulphuric acid was added, with great care, into approximately 800 cm^3 deionized water, in a fume-cupboard. After cooling the volume was made up to 1000 cm^3 with deionized water.

(iii) Polyacrylamide solution 0.05% m/V: 0.05 g of polyacrylamide was dissolved in approximately 80 cm^3 of deionized water, by stirring with magnetic stirrer for several hours. When the polymer was completely dissolved, it was diluted to 100 cm^3 by adding deionized water.

(iv) Standard phosphorus working solutions: 0, 1 and 5 µg P cm^{-3} solution were prepared in 0.5 M sodium bicarbonate extracting solution having pH 8.5.

2.7.4.2 Procedure:

The same procedure was adapted for preparation of soil extract, as in acetic acid extraction method (Section 2.7.1.2), except that 50 cm^3 of 0.5 M sodium bicarbonate solution was dispensed to each shaking bottle. The phosphorus concentration was measured colorimetrically, by Technicon Autoanalyzer II (Section 2.6.2.2.). 2 M sulphuric acid solution was used for neutralization of bicarbonate in a heating coil at 60°C. (manifold in Fig. 2.4)

2.7.5. Acidified ammonium fluoride (Bray-I reagent)

2.7.5.1. Reagents

(i) Acidified NH_4F (0.025 M HCl + 0.03 M NH_4F)

1. 1 M ammonium fluoride: 37 g Analar grade NH_4F was dissolved in deionized water and diluted to 1 litre.

2. 0.5 M HCl: 43.63 cm^3 of Analar HCl was carefully added to 800 cm^3 of deionized water, the volume was then made with water to 1 litre.

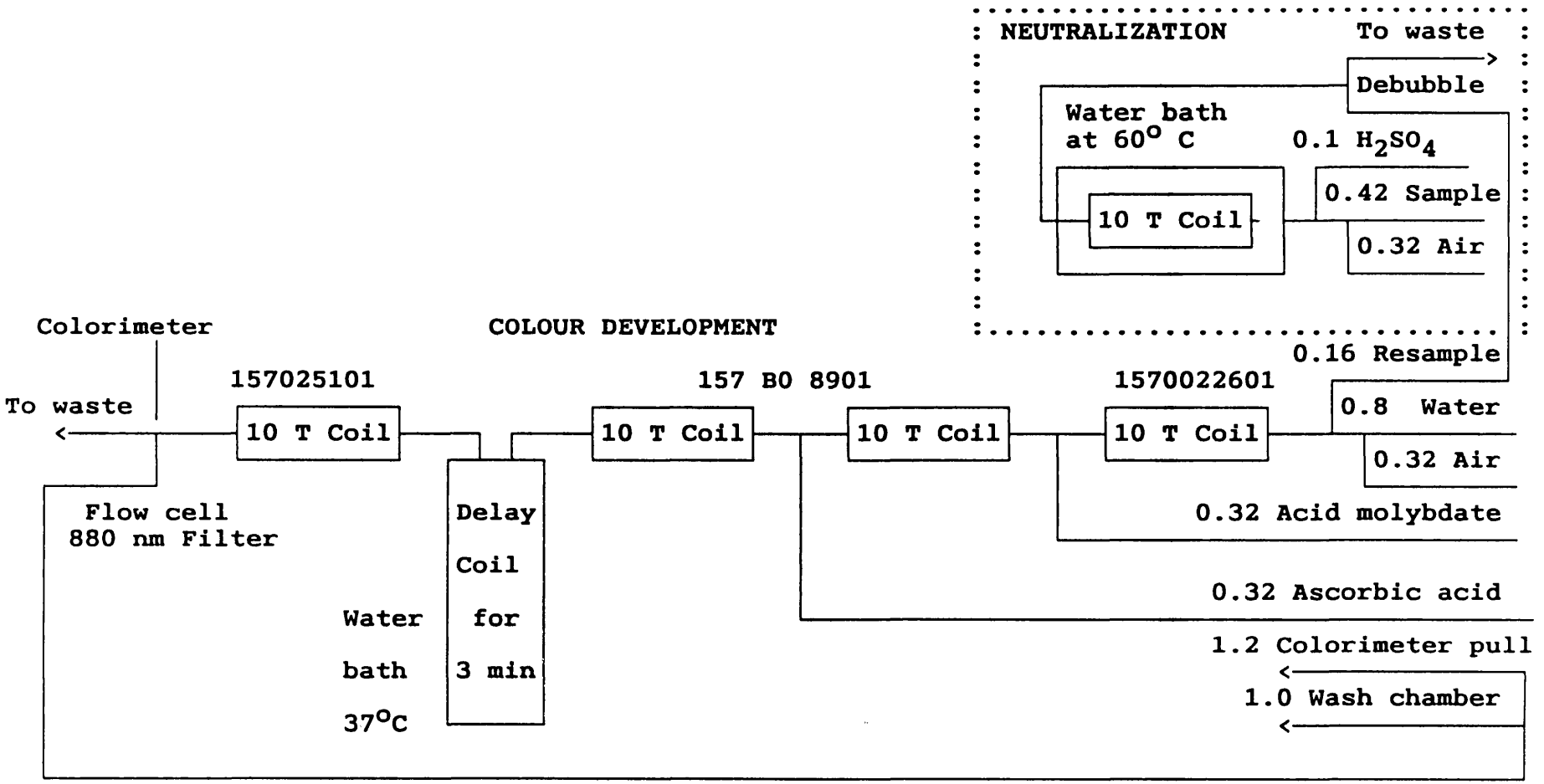


Figure 2.4 Technicon AutoAnalyzer II manifold for $NaHCO_3$ extractable P up to 5 µg cm^{-3}

3. Extracting solution (0.03 M NH_4F + 0.025 M HCl): 15 cm^3 of 1 M NH_4F and 25 cm^3 of 0.5 M HCl was added to 460 cm^3 deionized water.

(ii) Stock standard solution of phosphorus: 0.4394 g dried KH_2PO_4 was weighed accurately and dissolved in deionized water. The solution was diluted to 1000 cm^3 with deionized water. This solution contained 100 µg P cm^{-3}.

(iii) Working phosphate standard solution: 5 cm^3 and 10 cm^3 of the stock phosphate standard solution was diluted with extracting solution to 100 cm^3. This solution contained 5 and 10 µg P cm^{-3}, respectively.

2.7.5.2 Procedure

The same procedure was adapted for preparation of soil extract, as in acetic acid extraction method (Section 2.7.1.2), except that 50 cm^3 of acidified NH_4F extracting solution was dispensed to each shaking bottle. The phosphorus concentration was measured colorimetrically, by Technicon Autoanalyzer II (Section 2.6.2.2.).

2.7.6 Anion exchange resin method for extractable P:

The analytical method as presented by Amer et al. (1955) and Hislop and Cooke (1968) is time consuming and difficult to carry out on large scale. The major difficulties have now been overcome by placing the resin

in nylon mesh bags (Sibbesen, 1977). The method used herein was that of Sarirullah (1989) who followed the procedure of Sibbesen (1977).

2.7.6.1 Reagents

(i) Nylon mesh, 0.2 mm openings: Fine mesh nylon straining bags of 5 x 10 cm dimensions were prepared by folding and sewing the sides. After placing the resin in the bags, the bags were sealed and sewn.

(ii) Resin, Dowex IX8: The beads of the moist resin with 0.5 mm diameter were obtained by sieving. The coarse fraction which could not pass through a 500 micron sieve was used. 2.0 g resin was placed in each bag of nylon.

(iii) 1 M sodium chloride in 0.1 M HCl: 58.5 g Analar sodium chloride was dissolved in 900 cm^3 of 0.1 M HCl (8.73 cm^3 of concentrated HCl diluted to 1 litre) and the volume was made up to 1000 cm^3 with 0.1 M HCl.

(iv) Working standard phosphorus solution was made in the extracting solution (reagent iii).

2.7.6.2 Procedure

The bags of resin were placed in 4 oz screw capped shaking bottle, containing 50 cm^3 1 M sodium chloride (in 0.1 M HCl) and shaken for half an hour in an orbital shaker. The solution was discarded and the procedure was repeated twice more. The excess of chloride was removed

by washing with deionized water repeatedly until the decanted water was free of chloride (The presence of chloride in decanted water was tested with silver nitrate solution). The bags were shaken vigorously by hand for 2-3 seconds to remove excess water. The resin in the chloride form was ready for use.

The chloride resin bags were transferred to 4 oz shaking bottles, containing 2.5 g air dried soil and blanks (with out soil). 50 cm^3 deionized water was added to each bottle including blanks. The bottles were then shaken on an orbital shaker at 50 RPM for 24 hours. After shaking, the soil was poured out and the bags rinsed with deionized water. Phosphate adsorbed on the resin was then extracted by shaking with 50 cm^3 1 M sodium chloride solution (in 0.1 M HCl), for 30 minutes on orbital shaker. The concentration of phosphate was determined by the method described in Section 2.6.2.2. by using 5 µg P cm^3 made up in extracting solution as working standard solution.

The resin bags could be used again by treating with 1 M NaCl solution. The resin is regenerated in chloride form by repeating the whole process.

2.8 Phosphate adsorption and desorption:

2.8.1 Reagents

(i) Phosphorus stock solution

Approximately 20 g of KH_2PO_4 was dried in an oven at 110°C for one hour and then placed in desiccator for cooling. 8.788 g from the dried KH_2PO_4 was weighed accurately and dissolved in deionized water. The volume was made to 2 litre with deionized water. This solution contained 1000 µg P cm^{-3}.

(ii) Phosphorus working solutions

Working solutions of 1, 2, 5, 10, 20, 30, 40, 50, 70 and 100 µg P cm^{-3} were made by diluting 0.5, 1.0, 2.5, 5.0, 10.0, 15.0, 20.0, 25.0, 35.0 and 50.0 cm^3 respectively, from stock solution of 1000 µg P cm^{-3}. All the solutions were made in 500 cm^3 volumetric flasks.

2.8.2 Procedure

2.8.2.1 Phosphate adsorption

To obtain phosphate adsorption data for isotherm, 1 g of air dried soil passed through 2 mm sieve, from each soil was accurately weighed in 4 oz shaking bottles. 50 cm^3 of solutions containing 1, 2, 5, 10, 20, 30, 40, 50, 70 and 100 µg P cm^{-3} were added to the relevant bottles. The bottles were tightly capped and equilibrated by shaking in an orbital shaker for 24 hours at a constant temperature of 25°C. After equilibration the soil suspensions were filtered through Whatman filter paper No.2 in polyethylene bottles. Phosphorus concentration in the aliquots of the clear filtrate and original solutions

were measured on Technicon AutoAnalyzer II using the method described in Section 2.6.2.2. Two ranges 0-5 and 0-100 µg P cm^{-3} were used. For 0-5 range manifold in Fig. 2.1 was used, with sampling rate 50 samples per hour, whereas for 0-100 range dilution system was used (Fig. 2.2). 21-fold dilution was carried out with a sample flow rate of 0.1 cm^3 per minute and water flow rate of 2.0 cm^3 per minute. The sample rate in the dilution system was reduced to 40 samples per hour. The difference between the amount of phosphorus in the solution after shaking and the amount initially present was taken as the amount of phosphorus adsorbed by the soil.

2.8.2.2 Phosphate desorption

The phosphate desorption and adsorption experiments were started simultaneously. But after 24 hours shaking the bottles were again shaken for another 24 hours to desorb phosphate. In the desorption experiment 0.2 g air dried soil for higher dilution or 0.5 g soil for lower dilution was accurately weighed into 4 oz shaking bottles. 10 cm^3 and 25 cm^3 of 100 µg P cm^{-3} solution were added to the bottles respectively. After 24 hours of equilibration, deionized water was added to the bottles according to their dilution ratios. The bottles were again passed through the same process of shaking for 24 hours. The suspension was then filtered through Whatman filter paper No. 2. The phosphate concentration in the filtrate was measured in Technicon AutoAnalyzer II

(Section 2.6.2.2.) by using dilution manifold (Fig. 2.2). The phosphate concentration was measured in 0-100 µg P cm^{-3} range with 21-fold dilution as described in preceding Section. The amount of phosphate desorbed was calculated by the difference of the concentration of P added and the P concentration measured in the filtrate per gram of soil.

2.9. Digestion of herbage for phosphorus:

2.9.1 Reagents

(i) Sodium sulphate

(ii) Kjeldahl catalyst tablets (BDH): 1 tablet containing 1 g sodium sulphate and the equivalent of 0.05 g of selenium.

(iii) Concentrated sulphuric acid

2.9.2 Procedure

The same digestion procedure of herbage was used as followed by Shah (1988). Approximately 0.2000 g of finely ground herbage sample was weighed in an aluminium foil weighing boat, using subtraction method. The contents of the weighing boat were carefully transferred into the bottom of long narrow-necked Pyrex Kjeldahl digestion flask. 2 g sodium sulphate and 1 tablet of Kjeldahl catalyst were added to the flask to keep the ratio of sodium sulphate and selenium as 60 : 1. The addition of 2

g more sodium sulphate was to raise the boiling temperature of digestion mixture. 5 cm^3 of Analar concentrated sulphuric acid was added to the mixture in the flask carefully. The flasks were first heated gently on gas heated digestion stand to prevent frothing and then more strongly until the digest became clear. The flasks were then left at gentle heating with acid condensing one third way up the flask for about 2 hours. After cooling, 20 cm^3 water was added to each flask and swirled to dissolve the digest. This solution was then quantitatively transferred to a 100 cm^3 volumetric flask through whatman filter paper No. 40. The digestion flask was rinsed several times with deionized water and the aliquots added through the filter paper into the flask. The filter paper was also rinsed several times with deionized water through wash bottle. The digest was allowed to cool before making up the volume to 100 cm^3 with water. The samples were ready for the analyses of phosphorus, nitrogen and potassium contents in the herbage. The phosphorus concentration in the digest was determined colorimetrically by Technicon AutoAnalyzer II (Section 2.6.3.2), by using the manifold in Fig. 2.3.

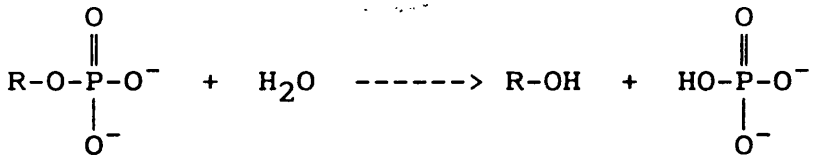
2.10. Assay methods of phosphatases:

The methods described here for assay of phosphomonoesterases and phosphodiesterase activities are based on the colorimetric estimation of the p-nitrophenol released by these enzymes when soil is incubated with

relevant buffered p-nitrophenyl phosphate solution. The incubation was carried out at 37^{o}C for 1 hour (Tabatabai, 1982).

2.10.1 Phosphomonoesterases (Acid and Alkaline phosphatases):

The general reaction for cleaving the phosphomonoester (substrate) by acid and alkaline phosphatases is given as follows:



2.10.1.1 Reagents:

(i) Modified universal buffer (MUB), pH 6.5 and 11.0:

For MUB 6.5, 2.42 g of tris(hydroxymethyl)aminomethane (THAM), 2.32 g of maleic acid, 2.8 g of citric acid, 1.26 g of boric acid and 3.904 g of NaOH were dissolved in approximately 800 cm^3 of deionized water in a beaker. The beaker containing the magnetic stirring bar was then placed on a magnetic stirrer. The solution was titrated with 0.1 N HCl to pH 6.5. The volume was then made accurately to 1 litre with deionized water.

For MUB 11.0 similar amounts of all chemicals mentioned for MUB 6.5 were dissolved in 800 cm^3 deionized water except the solution was titrated with 0.1 N NaOH to 11.0 pH and volume was then made to 1 litre.

(ii) p-Nitrophenyl phosphate solution, 0.025 M: 0.9279 g of disodium p-nitrophenyl phosphate hexahydrate (substrate) was dissolved in about 80 cm^3 of modified universal buffer (MUB) pH 6.5 (for assay of acid phosphatase) and pH 11.0 (for alkaline phosphatase) separately. Both of the solutions were made to 100 cm^3 with relevant MUB of the same pH. The solutions were stored in a refrigerator.

(iii) Calcium chloride 0.5 M: 18.375 g of $CaCl_2.2H_2O$ was dissolved in deionized water and the volume was made to 250 cm^3 with water.

(iv) Sodium hydroxide (NaOH), 0.5 M: 20 g of NaOH was dissolved in deionized water and the volume was made to 1 litre with water.

(v) Hydrochloric acid, 0.1 M: 25 cm^3 of commercially prepared 1 M HCl was diluted to 250 cm^3 in a volumetric flask.

(vi) Toluene Analar

(vii) Standard p-nitrophenol stock solution (1000 µg cm^{-3})

0.1 g of p-nitrophenol was dissolved in about 70 cm^3 of water in a volumetric flask. The volume was then made to 100 cm^3 with water and the solution was stored in a refrigerator.

(viii) Working standard solutions: 0.5 cm^3 from the stock solution of p-nitrophenol was diluted to 50 cm^3 accurately with respective MUB solutions. 0, 1, 2, 3, 4 and 5 cm^3 of diluted solution to various labelled vials and adjust the volume of each vial to 5 cm^3 by adding 5, 4, 3, 2, 1 and 0 cm^3 of MUB (pH 6.5 and pH 11.0) solutions. The volume in each vial was made up to 10 cm^3 by adding 5 cm^3 of 0.5 M NaOH (1 cm^3 of 0.5 M $Cacl_2$ + 4 cm^3 of 0.5 M NaOH were not added instead to avoid precipitation). This gave solutions of 0, 10, 20, 30, 40 and 50 µg cm^{-3} of p-nitrophenol.

2.10.1.2 Procedure

Duplicate 1.00 g of oven dry equivalent samples of fresh soil, 4 mm sieved were weighed in 15 cm^3 vials. 0.2 cm^3 of toluene, 4 cm^3 of MUB (pH 6.5 for assay of acid phosphatase or pH 11.0 for assay of alkaline phosphatase) and 1.0 cm^3 of p-nitrophenyl phosphate (PNP) solution made in the same buffer were added. The vials were swirled for a few seconds to mix the contents. Equal number of control vials were also processed in the same way except PNP (substrate) was not added to these vials before incubation. The vials were capped and were shaken on a shaker in an incubator for 1 hour at 37°C. After incubation the caps of vials were removed 1 cm^3 of 0.5 M $CaCl_2$ and 4 cm^3 of 0.5 M NaOH were added to the substrate treated samples first to stop the activity of the enzyme and then to the control vials. 1.0 cm^3 of the p-

nitrophenyl phosphate solution (substrate) was added to the control vials. The vials were swirled again to mix the contents. The soil suspension was filtered through Whatman No.2 filter paper. The intensity of yellow colour produced was then measured at 420 nm wavelength with a Bausch and Lomb spectrophotometer. In some soils dilution of 1:10 or 1:25 was made to bring the absorbence in measurable range.

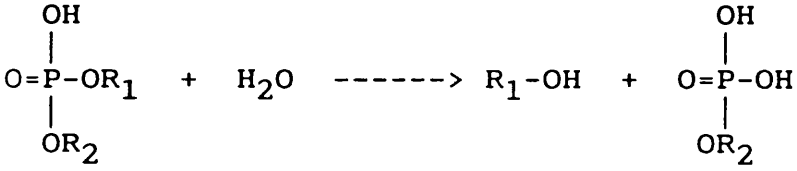
The standard graph was prepared from working standard solutions by plotting the absorbence against p-nitrophenol concentrations. Standard graph was made with every batch of samples.

The p-nitrophenol contents of the filtrates were calculated from the standard calibration graph.

As the time is an important factor in this procedure, so a little delay can cause a great difference in assay. Keeping in view the same fact volume of solutions was added with automatic pipette and maximum 20 vials were processed in a batch.

2.10.2 Phosphodiesterase

Phosphodiesterase catalyses the overall reaction of the type:



where R_1 and R_2 represent either alcohol or phenol groups or nucleosides.

2.10.2.1 Reagents

(i) Toluene Analar

(ii) Tris(hydroxymethyl)aminomethane (THAM) buffer, 0.05 M, pH 8.0: 6.1 g of THAM was dissolved in about 800 cm^3 deionized water. The solution pH was adjusted to 8.0 by titration with approximately 0.2 N H_2SO_4, the volume was made to 1 litre with water.

(iii) Bis-p-nitrophenyl phosphate (BPNP) solution, 0.005 M (substrate): 0.1811 g of sodium bis-p-nitophenyl phosphate was dissolved in about 80 cm^3 of THAM buffer pH 8.0, and the volume was diluted to 100 cm^3 with the buffer. The solution was stored in a refrigerator.

(iv) Calcium chloride solution 0.5 M: The solution was prepared as described for regent (iii) in Section 2.10.1.1.

(v) Tris(hydroxymethyl)aminomethane-sodium hydroxide (THAM-NaOH) extractant solution, 0.1 M, pH 12: 12.2 g of THAM was dissolved in about 800 cm^3 of deionized water. The pH of solution was adjusted to 12 by titration with 0.5 M NaOH, the volume was then made up to 1 litre with water.

(vi) Tris(hydroxymethyl)aminomethane (THAM) diluent, 0.1 M, pH ~10: 12.2 g of THAM was dissolved in about 800 cm^3 of deionized water, and the volume was made up to 1 litre with water.

(vii) Standard p-nitrophenol solution: The solution was prepared as described for reagent (vii) in Section 2.10.1.1.

2.10.2.2 Procedure

Duplicate 1.00 g oven dry equivalent fresh soil, 4 mm sieved was accurately weighed in 15 cm^3 vial. 0.2 cm^3 of toluene, 4 cm^3 of THAM buffer pH 8.0 and 1 cm^3 of BPNP solution were added. The vials were swirled for a few seconds to mix the contents. Equal number of control vials were also processed in the same way except BPNP (substrate) was not added to these vials before incubation. The vials were capped and were shaken on a shaker in an incubator for 1 hour at 37^oC. After incubation the caps of vials were removed, 1 cm^3 of 0.5 M $CaCl_2$ and 4 cm^3 of THAM-NaOH extractant solution were added to the all vials but to the substrate treated samples first to stop the enzymic activity. The vials were swirled again to mix the contents. The soil suspension was filtered through Whatman No.2 filter paper. The intensity of yellow colour produced was then measured at 420 nm wavelength with a Bausch and Lomb spectrophotometer. In some soils dilution of 1:10 or 1:25 was made with THAM pH ~ 10 (diluent) to bring the absorbence in measurable range. The p-nitrophenol content of the filtrate was calculated by reference to a calibration graph prepared from various standard

solutions of p-nitrophenol as described in Section 2.10.1.1.(vii).

2.11 Soil sterilization

2.11.1 Chloroform fumigation

The chloroform was purified before use for fumigation. Silica gel was first dried in an oven at 80^{o}C overnight. After cooling in a desiccator, it was put in three small beakers in a fume cupboard. 100 cm^{3} of Analar chloroform was poured into one beaker containing silica gel and the same treated chloroform was then in turn passed through second and third beaker. Purified chloroform was then collected in a fourth beaker. The fumigation of soil was done in a large desiccator lined with moist tissue papers. Approximately 50 cm^{3} of the purified chloroform was transferred to 250 cm^{3} beaker containing a few anti-bumping granules of silica gel. Beakers containing 20 g of soil to be fumigated and the beaker containing pure chloroform were put together in the desiccator. Air was evacuated from the desiccator until the chloroform started bubbling vigorously. The tap was closed and the desiccator was left in a dark cupboard for 20 hours. The beaker containing chloroform and the tissue paper were removed, fresh air was allowed into the desiccator for 5 minutes to take off the fumes of chloroform. It was then evacuated and this practice was repeated several times over a period of one and half hour

until the complete removal of chloroform vapour. The soil was stored in screw capped glass bottles for P adsorption studies.

2.11.2 Soil sterilization by autoclaving

Autoclave (manufactured by George T Gurr Ltd.) was filled with water up to a level where the bottom frame was sunk, was placed on burner ring. The empty vessel was placed in autoclave in its position. The heating of the autoclave was carried out on a bench covered with aluminium foil, to avoid damage to wooden bench.

When water started boiling, the glass bottles each containing approximately 20 g soil, covered with Al-foil were placed in the vessel and the lid of autoclave was put on and tightened. The soil samples were heated in the autoclave by maintaining the steam pressure to 15 lb/in^2 ($1kg\ cm^{-2}$) corresponding to 120^oC. After 30 minutes the burner was switched off and the samples were allowed to cool.

2.12 Collection and preparation of soil samples

Soil samples were taken in the fresh condition from the upper 0-15 cm depth for A horizon and 15-30 cm depth for B horizon. The samples in the labelled plastic bags were brought to the laboratory as soon as possible. The soil samples were stored in cold room at 2^oC. The samples were then spread on clean plastic sheets on the

laboratory tables and air dried to the extent to pass through 4 mm sieve. Half of the each sieved soil sample was stored in cold room while the remaining half was spread on plastic sheets and left at room temperature until completely air dried.

Some of the samples were collected from various locations in England (3 soils from Midelney Series, Swaffham Prior series and Alluvium) but most of the soil samples were collected from different locations of Scotland. The system of classification of Soil Survey of England and Wales is different from that of Scotland. The Soil Survey of England and Wales classifies soils into Series. A Series is a uniform soil forming on a particular parent material. The Soil Survey of Scotland classifies soils into Soil Associations. The Soil Associations are further subdivided into Series on the basis of point in a catena and drainage status. The Soil Association is the catenary sequence of soils on a particular parent material. Soil Series within the Association are distinguished on the basis of drainage status.

2.13 Soil sampling sites

A brief description of each of the soil sampling site is given below. Soils were attributed to Soil Series using the Soil Memoirs and Soil Maps for each area. Where the area had not been mapped , Soil Memoirs for an

adjacent area were used. (Mitchell and Jarvis, 1956; Grant et al., 1962; Seale, 1975; Ragg et al., 1976)

Dreghorn No.1

The site is situated at West of Scotland College of Agriculture, Auchincruive, Ayr, Scotland. Grid reference No. is NS 373232. The soil is under permanent grass adjacent to greenhouses. It belongs to the Dreghorn Association which is developed from raised beach deposits. The Series is Dreghorn which has been classed as freely drained brown forest soil.

Caprington

The site is situated at West of Scotland College of Agriculture, Auchincruive, Ayr, Scotland. Grid reference No. is NS 376232. The soil is devoted to permanent grass and managed for grazing by dairy cows. The soil belongs to Rowanhill Association which is developed from glacial till derived from sandstone and shale of the productive coal measures. The soil comes under the Caprington Series which is classed as an imperfectly drained brown forest soil.

Dreghorn No. 2 (Arkleston)

The soil is located at Arkleston Farm, Paisley, Scotland. The Grid reference No. is NS 508655. The soil is used for intensive cultivation of potatoes, barley and oil seed rape. It belongs to Dreghorn Association which

is formed from raised beach deposits. The Series is Dreghorn which has been classed as freely drained brown forest soil.

Dunlop

The site is situated at South Drumboy Farm, Fenwick, Ayrshire, Scotland. The Grid reference No. is NS 500484. The soil is under permanent grass and used for sheep grazing and beef cattle. The site receives occasional dressing of lime. The soil belongs to Darleith Association which is developed on till derived from Carboniferous age igneous rocks (Basalt). The Soil Series is Dunlop which is classed as an imperfectly drained brown forest soil.

Darvel

The site is located at Westerton Farm, Lennoxtown, Scotland. Grid reference No. is NS 635773. The soil is devoted to permanent grass and rough grazing. It belongs to the Darvel Association which is formed from fluvioglacial sands and gravels derived from Carboniferous igneous and sedimentary rocks. The soil comes under the Darvel Series which has been classed as freely drained brown forest soils of low base status.

Bargour

The site is situated at West of Scotland College of Agriculture, Auchincruive, Ayr, Scotland. Grid reference

No. is NS 379234. The soil is under permanent grass just near to the meteorological station. It belongs to Bargour Association which is developed from till derived from Carboniferous sandstone. The Series is Bargour which has been classed as imperfectly drained brown forest soil.

Peat (Blackstoun)

The site is situated at Blackstoun Scotland. Grid reference No. is NS 449661. The soil was reclaimed by drainage. The soil is used for intensive cultivation of potatoes and barley. The soil has not been classified in any soil association.

Midelney (Grass)

The site is located at Bank Farm, Norfolk, England. The Grid reference No. is TF 588022. The soil was ploughed out for permanent grass in 1979. Now it is used for intensive cultivation of arable crops. It belongs to Midelney Series which is formed from calcareous alluvial clay parent material. The Series has been classed as a ground water gley.

Midelney (Arable)

The site is located at Bank Farm, Norfolk, England. The Grid reference No. is TF 588022. The soil is used for intensive arable crop production e.g., wheat, potatoes and sugar beet. It belongs to Midelney Series which is

developed from calcareous alluvial clay parent material. The Series has been classed as a ground water gley.

Alluvium

The site is located at Bank Farm, Norfolk, England. The Grid reference No. is TF 588022. The soil is cultivated as garden. It is originated from calcareous river alluvium produced by dredging the mud from the river Great Ouse in 1961.

Swaffham Prior

The site is located at , England. The Grid reference No. is TL 720948. The soil is used for arable crop production e.g., sugar beet. It belongs to Swaffham Prior Series which is developed on a thin chalk-loam drift. Solid chalk occurs within 2 m. It is classed as well drained calcareous soil.

2.14 Soil Properties

Routine soil properties are given in Table 2.2 and 2.3

Table 2.2 Particle size distribution of soils

SOILS	%Clay	%Silt	%Fine sand	%Coarse sand	Textural Class
Dreghorn No.1 A	4.5	12.4	25.9	49.7	Sandy loam
Dreghorn No.1 B	5.4	8.2	27.2	58.8	Loamy sand
Darvel A	15.8	29.4	20.5	32.2	Sandy clay loam
Capring-ton A	18.1	27.8	21.5	30.4	Sandy clay loam
Bargour A	10.6	24.7	21.5	42.2	Sandy loam
Dunlop A	27.1	35.2	11.3	25.5	Clay loam
Peat(Bla-ckstoun)	23.3	45.7	19.0	10.1	Clay loam
Dreghorn No.2 A	12.6	28.4	31.3	26.9	Sandy loam
Dreghorn No.2 B	10.8	25.2	34.7	28.7	Sandy loam
Midelney (Grass) A	38.6	42.3	15.0	3.0	Clay
Midelney (Arable) A	41.0	47.3	9.1	2.4	Silty clay
Midelney (Arable) B	48.4	47.0	2.6	0.5	Silty clay
Alluvium A	16.8	41.2	37.8	1.6	Sandy silt loam
Swaffham Prior A	9.4	29.4	22.4	36.7	Sandy loam

Coarse sand > 0.18 mm, Fine sand < 0.18-0.05 mm, Silt < 0.05-0.002 mm, and Clay < 0.002

Table 2.3 Soil properties

SOILS	pH H_2O	pH $CaCl_2$	LOI %	O.C. %	Fe %	Al %
Dreghorn No.1 A	5.2	4.5	4.8	1.84	0.29	0.33
Dreghorn No.1 B	5.5	5.0	2.2	0.68	0.25	0.36
Darvel A	5.7	5.3	8.8	2.95	0.85	0.42
Capring-ton A	5.9	5.3	8.4	3.41	0.52	0.17
Bargour A	5.5	4.9	5.8	1.87	0.63	0.35
Dunlop A	4.9	4.4	18.4	6.69	1.08	0.58
Peat(Bla-ckstoun)	5.4	4.9	20.8	8.41	1.11	0.45
Dreghorn No.2 A	5.8	5.4	8.8	3.51	0.96	0.43
Dreghorn No.2 B	6.1	5.6	5.0	1.20	0.67	0.35
Midelney (Grass) A	6.3	6.0	15.6	6.18	0.74	0.11
Midelney (Arable) A	7.2	6.6	11.4	4.19	0.59	0.11
Midelney (Arable) B	7.6	7.2	5.8	1.67	0.33	0.11
Alluvium A	7.4	7.0	6.2	2.78	0.41	0.08
Swaffham Prior A	7.9	7.3	3.2	1.28	0.11	0.10

LOI = Loss on ignition, O.C. = Organic carbon

CHAPTER 3

ESTIMATION OF EXTRACTABLE SOIL PHOSPHATE

3.1. Introduction

Because of the increasing cost of fertilizer materials and the desire for environmentally correct usage of fertilizers, there is a renewed interest in developing sound systems for testing soils to make fertilizer recommendations.

3.1.1. Analytical soil P testing procedures

Methods for determining soil phosphorus and its availability to plants are essential for developing principles and knowledge of the nature and behaviour of P in soils. The purpose of these methods has been to characterize the P in soil system. Soil testing procedures are used, with varying degree of success, to determine the P status of soils and to assist in making fertilizer recommendations. A major limitation of such procedures is the results obtained are influenced by soil type with a result that extensive field calibration is often required (Luscombe et al., 1979). The quantities and origin of P removed by the many extraction procedures vary widely. The pH of the extraction particularly has substantial effects on the amount and form of P removed. A criticism of extractants that are either more acidic

or alkaline than the soil solution is that a portion of the P extracted is of low plant availability. Conventional methods for the determination of available phosphorus appear to extract a portion of all chemical forms having either high solubility or high specific surface (Chang and Jackson, 1957). Four factors have been described by Mendoza and Barrow (1987) to determine the phosphate status of a soil: the amount of native phosphate originally present in the soil in a form that would available to plants; the amount of phosphate that has been added as fertilizer or by mineralization of organic matter less that lost in exported products ; the period that has elapsed since the phosphate was added ; and the ability of the soil to retain phosphate.

Yong and Bartlett (1977) emphasized three pieces of information, which must be furnished for making P fertilizer recommendations based on soil test results: the present intensity of P in a particular soil ; the optimum intensity level for a particular crop on that soil; and the amount of fertilizer required to increase the present level to the optimum level. It is recognized that this amount of fertilizer varies with the capacity of a soil to fix P. It was hypothesized that the quantity of fertilizer P needed will be a function of the soil test P intensity sought and that the slope of P quantity vs. P intensity will be predictable from soil characteristics such as extractable Al, pH, lime-

requirement-buffer pH, soil organic matter, or clay content.

The main source of plant-available phosphorus is generally termed the labile pool. This provides fairly rapid exchange with soil solution, maintaining the solution concentration. The remaining fraction is the non-labile pool. This contains the large quantity of insoluble phosphate which is only very slowly released into the labile pool. The various organic and inorganic phosphates constitute these labile and non-labile pools. There is no clear distinction by which particular forms can be designated as in one or other pool. In general the labile pool can be considered as orthophosphate adsorbed on the surfaces of clay minerals, hydrous oxides and carbonates plus iron and aluminium phosphates. The relationship between the quantity of phosphorus in the labile pool and the soil solution concentration depends particularly on soil texture and pH (Archer, 1988). Adsorption of orthophosphate is stronger and therefore phosphate is less available at low pH. Precipitation as iron and aluminium phosphates will also reduce phosphorus availability. In calcareous soils, precipitation of calcium phosphate is the major factor limiting solubility. Phosphate adsorbed on calcium carbonate will slowly convert to the mineral, apatite. Phosphate availability is generally greatest between pH 6.0 and 7.0.

It has been claimed that the best criterion for a P-test is whether it corresponds to the crop yield response to P-addition. However, there is unlikely to be a direct relation between P-test values and the crop yield response to P-addition. Sibbeson (1977) reported that P-test only provides a relative estimate of an ill-defined parameter called the P-status. This may be thought of as the ability of a soil to release P to a crop during growing season ; or specifically , as the ability of a soil to release P to a given plant root for a given period under optimum conditions with respect to all other growth factors. Apart from the soil P-status, the crop yield response to P-addition depends on other factors such as the chemical, physical and biological soil properties, the climate and the type of fertilizer. P-test, therefore, should be evaluated by comparing their P-test values to P-uptake of plants (Mendoza,1989). Such evaluations should include many different soils, and they should be made under well controlled conditions with the soil P as the only limiting growth factor. Estimation of available soil phosphorus of soil is necessary for: Phosphatic fertilizer recommendations (Walmsley and Cornforth,1973); growth response to the application of fertilizer phosphorus (Ipinmidun,1973); and the amount of phosphorus a soil can supply.

3.1.2. Grouping of soils

Soils have been grouped into three categories for the purpose of fertilizer recommendations, low, medium and high levels of test phosphorus (Fitts and Nelson, 1956). Scaife (1968) and SAC (1985) classified soils into five categories, on the basis of standard phosphate requirements (Juo and Fox, 1977). while MAFF (1983) has ten categories of phosphorus levels from agricultural land. Sharpley (1991) divided soils into three groups based on taxonomic and chemical characteristics. Beckett and White (1964) reported that the phosphate potential of a given soil is controlled by the relative amounts of labile P and Ca. They suggested that isotopically exchangeable P of a field soil must be divided functionally into four parts: P at surface nett-exchange sites, not immediately labile - requiring only to be displaced by OH^- or possibly another anion to be available for uptake ; P held at occluded nett-exchange sites, not immediately available but capable of mobilisation as a result of slow counter-diffusion and exchange of $H_2PO_4^-$ and OH^-, in response to the depletion and consequent lowering of phosphate activity in soil solution ; P held at surface sites, on crystalline phosphates not to be exchangeable by hydroxyl, but nevertheless capable of moderately rapid mobilization - this fraction will be particularly sensitive to the chelating action of root and microbial exudates ; P held

within more perfect crystal lattices at sites from which P can only be mobilised both by disposal of complementary cations and by substantial crystal arrangements, this fraction is available only to prolonged cropping.

Most soil phosphorus determinations have two distinct phases-first, the preparation of a solution containing the soil phosphorus or fraction thereof, and second, the quantitative determination of phosphorus in this solution (Olsen and Sommers, 1982).

From the time Dyer (1894) introduced the use of citric acid solution, soil scientists have endeavoured to extract from the soils a fraction of the soil phosphate that can be considered to be available to plants. The basis of this technique is the measurement of the quantity of phosphate dissolved when soil is treated with an extracting reagent. A good soil extractant should extract all the available form or forms of a nutrient from soil with specific properties. There should be a minimum secondary precipitation and adsorption of extracted phosphorus.

3.1.3. Conventional soil P extraction methods

Soil P extractants can be categorized into various groups on the basis of their chemical nature. In certain cases anions in the solution may have the specific effect either because of their anion replacing ability or their reaction with the cations associated with the phosphorus.

A list of the commonly used extractants to measure the available phosphorus is given below:

3.1.3.1. Dilute concentration of strong acids

The common strong acids used for extracting the phosphorus are hydrochloric acid (HCl), sulphuric acid (H_2SO_4) and nitric acid (HNO_3). The concentration of acids varies from 0.002 M to 0.07 M. The pH of the dilute solutions generally range from 2 to 3. The nitrate and chloride ions have very little effect on the extraction, but sulphate tends to reduce readsorption of the phosphorus dissolved by the acid solution (Kamprath and Watson, 1980). Chang and Jackson (1957) found, in isolating the chemical forms of inorganic phosphate, that 0.5 N sulphuric acid dissolves Ca-phosphate (apatite) completely and aluminium phosphate and iron phosphate considerably. The mixed acids (0.05N HCl + 0.025N H_2SO_4) method for extracting phosphorus has been regarded more effective than HCl alone, by Nelson et al. (1953).

3.1.3.2. Dilute concentration of strong acid plus a complexing ion

The complexing ion used most often, in extracting phosphorus, is fluoride. It is the most highly electronegative of all anions and is considered to be specifically adsorbed to replace the adsorbed phosphate (Bower and Hatcher, 1967). Fluoride forms strong complexes with the aluminium Al^{3+} ions, thus releasing P

from Al-P (Kamprath and Watson, 1980). The active fluoride ion coupled with the strong acidity makes this reagent capable of extensive attack on all forms of inorganic P in soil. The fluoride compound generally used is NH_4F. The most common extracting solutions of this kind are mixture of HCl and NH_4F. The combination of HCl and NH_4F remove easily acid-soluble P forms, largely calcium phosphates, and a portion of aluminium and iron phosphates. The method proposed by Bray and Kurtz (1945), in general, has been most successful on acid soils, which extracts mineral phosphates of aluminium and to a lesser extent iron (Holford and Crocker, 1988). Bray P-I reagent (0.03N NH_4F + 0.025N HCl) decreased the mean extractable P from 306 to 37 mg/kg in 12 calcareous soils, but the same reagent increased extractable P (Manikandan and Sastry, 1986), threefold to fourfold from Al and Fe phosphates (Olsen and Sommers, 1982).

Neutral ammonium fluoride is also used as an extractant, which determines adsorbed phosphate. Strong complex formation between fluorine and aluminium make this reagent a powerful extractant of aluminium-bound P (Williams and Knight, 1963). Turner and Rice (1952) found that neutral ammonium fluoride can dissolve aluminium-phosphate but not iron-phosphate. Chang and Jackson (1957) reported in fractionation of soil phosphorus that neutral ammonium fluoride in a single extraction dissolves aluminium phosphate completely, iron phosphate

slightly and apatite negligibly, in a soil extractant ratio of 1:50. NH_4F in calcareous soil or sometime in acid soil, reacts with $CaCO_3$ with the formation of CaF_2 causes over-estimation of acid extractable Ca-phosphate, and under-estimation of non-occluded Fe and Al bound phosphates. F^- ions are very effective in complexing Al^{3+} ions and in this way causes releasing of phosphorus from Al-bound P. Calcium is precipitated by F^- ions and therefore the phosphorus present in the soil as $CaHPO_4$ will be extracted by solution containing F^- ions (Thomas and Pealsee, 1973). Al^{3+} ions have greater affinity for F^- ions (Lindsay,1979).

3.1.3.3 Dilute concentration of weak acids

The most common weak acids used for extracting soil phosphorus are citric acid, lactic acid and acetic acid. Salts of weak acids have also been used as soil-P extractants. The organic anions can replace adsorbed phosphorus and prevent its readsorption. Acetic acid, alone or buffered by acetate salts, has been used for a long time to measure the status of plant nutrients in the soil. 0.43M (2.5% v/v) acetic acid is the most commonly used soil P extracting solution by the Advisory Service in Scotland. Acetic acid solution, which has generally a pH 2 to 3, provides sufficient hydrogen ion activity to dissolve calcium phosphate. It will also solublize some of the aluminium and iron phosphate. Egner-Rheim's

reagent (ammonium lactate/acetic acid, pH 3.8) has also been used in extracting soil P.

3.1.3.4 Acetate buffers (Morgan and modified Morgan)

Both Morgan's reagent (3% acetic acid buffered with 10% sodium acetate) and modified Morgan's (3% acetic acid buffered with 4% ammonium acetate) have pH 4.8, extract relatively small amounts of phosphorus as compared with acetic acid (Reith et al., 1987). Acid ammonium acetate was effectively used in extracting soil P. It breaks up the structure of aluminium oxide polymers and probably also extracts some of the phosphorus which is not labile (Aura, 1978).

3.1.3.5 Buffered alkaline solution

The choice of 0.5 M solution of sodium bicarbonate by Olsen et al. (1954), as a soil test for P estimation in calcareous soil, was based mainly on considerations of the solubility of phosphate. However, it has also been commonly used as a soil P extractant in acid and neutral soils. It is thought that sodium bicarbonate removes phosphorus by two mechanisms: anions replacement and hydrolysis of cations bound phosphorus. In the first mechanism, phosphorus adsorbed on surfaces of $CaCO_3$ and hydrated oxides of iron and aluminium can be replaced by bicarbonates (Olsen et al., 1954). In the second process, $NaHCO_3$ extracts phosphorus from Al-P and to some extent

Fe-P due to hydrolysis of Al^{3+} and Fe^{3+} (Tyner and Davide, 1962).

The HCO_3^- ion is quite effective in replacing adsorbed phosphorus, and reduces the activity of Ca^{2+} in solution by causing precipitation of Ca^{2+} as $CaCO_3$. As a result the concentration of phosphorus in solution is increased. In acid soils containing iron and aluminium phosphates, phosphorus concentration in solution increases as the pH rises (Lindsay and Moreno, 1960 ; Naidu et al., 1987). By increasing soil pH, the availability of P is increased because it inhibits the adsorption-precipitation reactions between P and Fe or Al and also accelerates organic matter decomposition (Adams and Odom, 1985). The mild alkaline bicarbonate, in acid soils reflect largely the labile pool of exchangeable, aluminium- and iron-bound P available to plants (William and Knight, 1963). The Olsen's reagent desorbs loosely held P and some chemisorbed P (Griffin, 1971). Secondary precipitation reactions in acid and calcareous soils are reduced to a minimum because the concentration of Al^{3+}, Fe^{3+} and Ca^{2+} remains at low level in this extractant (Olsen and Sommers, 1982). Sodium bicarbonate extracts more phosphorus than acetic acid from the same soil, because it extracts phosphorus from soil as physisorbed, chemisorbed and also a small amount of microbial phosphorus (Bowman and Cole, 1978). Barrow and Shaw (1976) pointed out that secondary reactions are minimised

by the use of sodium bicarbonate as a soil test. They enumerated the factors which affect their extent: the soil:solution ratio, the period of shaking with bicarbonate, the kind of soil, and the level of phosphate. Since Olsen's extractable phosphorus was much less dependent on soil type, it was considered as more suitable index of plant available phosphorus in soils differing in phosphorus retention (Smith and Gregg, 1982). The two sodium bicarbonate tests (Olsen et al., 1954 ; Colwell, 1963) are commonly used in Australia (Holford and Cullis, 1985 ; Mendoza and Barrow, 1987). The experience in Britain is that extraction of soil with sodium bicarbonate is generally the best method on which advice to farmers on fertilizer use, can be based (Wild, 1988). Recently Olsen's test has been recommended as routine method for determining available soil phosphate, by ADAS, in soils of England and Wales. Fardeau et al. (1988) suggested that Olsen's method is the best method for assessing available soil phosphorus both in calcareous and acid soils.

3.1.3.6 Anion exchange resin

A method for characterizing soil-P, the use of anion exchange resin, was originally developed by Amer et al. (1955), who described its action more nearly analogous to plants. The removal of phosphate from soil by plants or by an anion exchange resin involves the following process:

P (solid)--> P (solution)--> P (plant or resin) (Non-labile + Labile)

Phosphate depletion either by plants or anion exchange resin did not change the phosphate potential of a soil (Elrashidi and Larsen, 1978). They thought that some of the labile phosphate removed by the plants was replaced by a mobilization of non-labile phosphate in the soil. The resin removes phosphate from solution, but would not return another phosphate ion in exchange. Anion exchange resin extract P without chemical alterations or pH changes. Crisanto and Sutton (1973) regarded it the quickest and easiest method. It stimulates the desorbing effect of plant roots better than the usual chemical extractants (Bache and Ireland, 1980). Cooke and Hislop (1963) considered, the anion exchange resin method, a very suitable method for routine examination of soil P, which avoid chemical destruction of soil. Under appropriate conditions, the rate of uptake of phosphate by the resin is only dependent on the rate of release of phosphate from the soil and not on the properties of the resin. Actually the resin functions as a plant root with a very high capability for phosphorus uptake. Unlike the plant root the resin, however, releases chloride or bicarbonate ions for the anion extracted. Roots secrete exudates like organic acids, amino acids and sugars, which help in solublizing phosphorus. The amount of soil P desorbed by anion exchange resin has been found to be

closely correlated with P uptake by plants (Amer et al., 1955; Cooke and Hislop, 1963; Sibbesen, 1978). Hislop and Cooke (1968) pointed out following factors which may influence the amounts of phosphate extracted from soil by anion exchange resin method: (a) Relative volumes of soil, resin and water (b) Available surface area of soil and resin (c) Temperature of the suspension (d) Duration and vigour of shaking. Later on two more factors: the type of anion exchange resin and the anionic form of resin were added by Sibbesen (1978). He recommended that resins in bicarbonate form should be used for both routine as well as more advanced analyses of the ability of soils to supply phosphate to plants. Bache and Ireland (1980) reported that extraction of P by anion exchange resin depends on two reactions: uptake of P by the resin from solution, and the release of P from soil. They also compared HCO_3-resin with Cl-resin in desorption of phosphate from soils. They found that P extracted by HCO_3-resin was much greater than by Cl-resin from an acid soil, due to lower total anion and higher desorbing anion concentration, but there was little difference between two resins with a calcareous soil. All these factors controlling the P desorption by anion exchange resin are interdependent (Dalal, 1985).

Although extracting P by anion exchange resin is superior to the other extracting methods for predicting the response to P, it is more time consuming, suitable

for single measurement (Bache and Rogers, 1970; Khalil, 1981; SAC, 1985). Reith et al. (1987) regarded anion exchange resin, the best method, while comparing the correlation coefficients for five extractants i.e. Acetic acid, Morgan's reagent, Bicarbonate, Lactate and Resin for estimating the soil phosphorus in different soil series of Scotland.

3.1.4 Selection of suitable extractant

A knowledge of relationship between soil test methods and phosphorus fractions and plant parameters helps in selecting a suitable soil test method for a particular group of soils. The most appropriate soil test method for a soil would be one which extracts predominantly that fraction of phosphorus which plays a major role in plant uptake. Many methods have been used by various researchers, for assessment of plant available phosphorus. Most of the methods based on extraction procedures differ according to the nature, concentration, composition and pH of the extractant used, soil-extractant ratio, time and methods of shaking, and temperature. These determine the strength of the extraction and the extent to which the value can be regarded as reflecting capacity or intensity. Weak reagents, such as water and neutral salts, reflect the intensity of the soil P, very little being actually extracted. Other reagents, such as strong acid or alkaline solutions, extract large amounts of P and give

predominantly quantity measurements. Between these extremes, there is range of conventional methods of varying extracting power, which may give a composite index of quantity and intensity. In extracting procedure, the P in solution is in equilibrium with P on soil surfaces, in the soil suspension. Depending on the extraction method and the type of soil, the readily soluble P may be the only a small portion of the total P. For a given method, variation from one soil to another indicates differences in the strength of P retention. Such differences correspond to the variations in the intensity of P supply to plants. The extractable P by an extractant should reflect the intensity factor. As there can be no universal method of extraction for all soil types, hence the parameters of quantity, capacity and intensity should be considered in assessing available soil phosphorus (Fardeau et al., 1988). The selection of a suitable method depends on the objectives that require the soil phosphorus measurement. In selecting a suitable method for P extraction following points may be considered: (a) The amount of phosphorus extracted should correlate with plant uptake and yield response to added phosphorus. (b) There should be a minimum of secondary precipitation and adsorption of extracted phosphorus. (c) The method should be adaptable to rapid, routine analysis. (d) The method adapted should be applicable to the specific type of soil sample under study.

The present study was conducted to evaluate, the relative performance of various commonly used extractants, on different soils.

3.2 Materials and methods

3.2.1 Soils

Fourteen soil samples were collected at random from various sites in UK. The detail of these sites have been given in Chapter 2 (Section 2.13).

3.2.2 Extraction methods for soil phosphate

Six different procedures were selected for extraction of soil phosphate. These procedures are widely used, for estimation of available soil phosphate, in the domain of agriculture. All procedures for getting soil extracts, were applied to soil samples in the same manner, without modification from the original, published instructions with respect to soil:solution ratio, shaking time and nature of reagents. All soils were extracted in triplicate with each extractant. The procedures followed in this study have already been described in Section 2.7 in detail.

3.3 Results and discussion

3.3.1 Estimation of soil phosphate by extractants

The mean amounts of PO_4-P extracted from the soils by the six extractants are given in Table 3.1. As expected,

Table 3.1 Extractable phosphate in soils by different extractants(µg g-1 soil)

Soils	----------E X T R A C T A N T S---------- NH_4-acetate	Na-acetate	Acetic acid	Na-bicarbonate	Bray-I	AER
Dreghorn 1A	5.3	6.2	8.9	15.9	68.5	7.1
Dreghorn 1B	0.5	1.4	1.5	4.2	31.7	1.4
Darvel A	11.2	13.6	11.5	32.3	114	11.9
Caprington A	15.8	14.3	27.2	28.2	64.5	19.9
Barg-our A	22.6	25.6	29.1	57.2	211	28.5
Dunlop A	5	8.3	11.9	26.4	127	4.5
Peat(Blackstoun)	8.8	12.7	21.4	38.4	140	8
Dreghorn 2A	9.5	10.4	23.7	25.2	153	6.4
Dreghorn 2B	0.7	1.6	0.8	5.2	17.8	0.3
Midelney (Grass)	51.6	44.3	112.0	34.9	93	28.3
Midelney (Arable)	32.4	29.1	69.0	27.0	56.9	13.8
Midelney (Arable)B	11.6	3.5	49.7	2.0	2.7	1.9
Alluvium	112	113.0	113.0	81.0	0.8	20.1
Swaffham Prior	80	68.5	133.0	37.9	0.6	12.5
Means	26.3C	25.2C	43.8B	29.7C	77.2A	11.8D

Means followed by similar letter(s) do not differ significantly at 1% level of probability.

the amounts of phosphate extracted showed a wide variation between soils.

A statistical analysis of data showed that the amount of PO_4-P extracted by various extractants varied significantly. This was expected because the extracting solutions tested, extracted different forms of phosphorus (Bray and Kurtz, 1945; Nelson et al., 1953; Olsen and Sommers, 1982). The highest PO_4-P was removed by Bray-I reagent, followed by acetic acid, which indicates that these methods removed some of the unavailable P (William and Knight, 1963).

The performance of sodium bicarbonate, sodium acetate and ammonium acetate was comparable. This suggests that the three methods probably extract some of the same kind of soil P (Radwan et al., 1985). The lowest amount of PO_4-P was recorded in anion exchange resin, which is analogous to plant root.

3.3.2. Correlation between extractants for estimation of extractable soil phosphate

Correlation coefficients between six extractants, for all 14 soils studied are presented in Table 3.2. It was observed that Bray-I reagent showed non-significant correlation with all the other extractants under study. The relationship of Bray-I test was affected by soil characteristic, particularly by oxalate extractable Al (Giroux and Tran, 1985). The Bray-I procedure performed erratically in calcareous soils as compared to others in

Table 3.2 Correlation coefficients between extractable soil phosphate using six extractants from 14 soils.

EXTRACTANTS	NH_4-acetate	Na-acetate	Acetic-acid	Na-bicar-bonate	Bray-I
Na-acetate	0.99***				
Acetic acid	0.90***	0.85***			
$NaHCO_3$	0.75**	0.81***	0.53NS		
Bray-I	-0.38NS	-0.32NS	-0.38NS	0.23NS	
Anion ex-change-resin	0.54*	0.55*	0.53NS	0.73**	0.32NS

NS = Non-significant at 5% * = Significant at 5%

** = Significant at 1% ***= Significant at 0.1%

the study. Similar results were obtained by Van Lierop and Tran (1990). The three extractants: ammonium acetate buffer, sodium acetate buffer and 0.5M sodium bicarbonate revealed best relationship, in all the 14 soils. This suggests that these extractants have similarity in selectivity of dissolution of same kind of phosphates (Chang and Juo, 1963). Anion exchange resin showed better correlation with sodium bicarbonate, good correlation with ammonium acetate buffer as well as with sodium acetate buffer and non significant correlation with other two extractants.

Sodium bicarbonate also behaved like anion exchange resin, in having no significant correlation with acetic acid and acidified ammonium fluoride (Bray-I reagent). However, it indicated better correlation with ammonium aetate buffer and anion exchange resin.

Keeping in view the general trend of soils in the extractable phosphate relationship (Fig 3.1 to 3.3), the soils were split into two groups, on the basis of pH. Re-calculated coefficients of correlation are given in Table 3.3 and 3.4. It was observed that correlations between extractants in soils having pH below 6.3 were improved (Table 3.3). The results are consistent with the findings of Van Lierop (1988), who obtained significant correlations between P values extracted from acid soils with either Bray-I P or 0.5N $NaHCO_3$ and 0.25, 0.5 or 1.0N Acetic acid solutions. But the correlations between

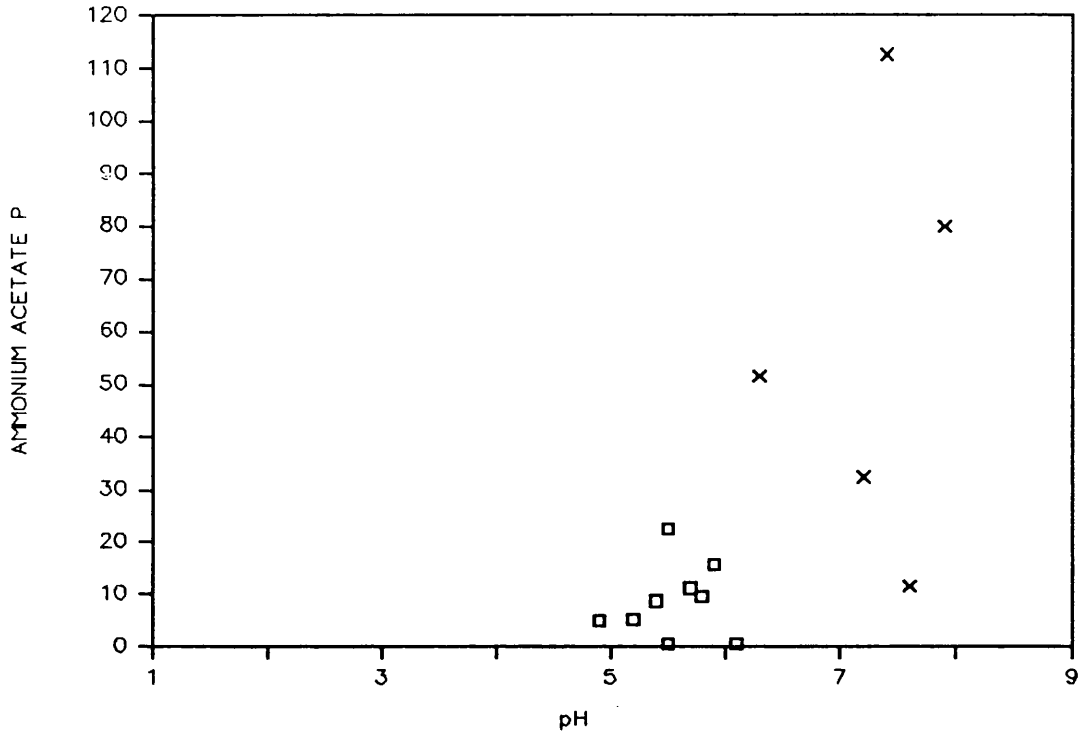


Fig 3.1 Scatter diagram showing the relationship between ammonium acetate extractable P and pH of the soils.

(□) Soils with pH < 6.2

(x) Soils with pH > 6.2

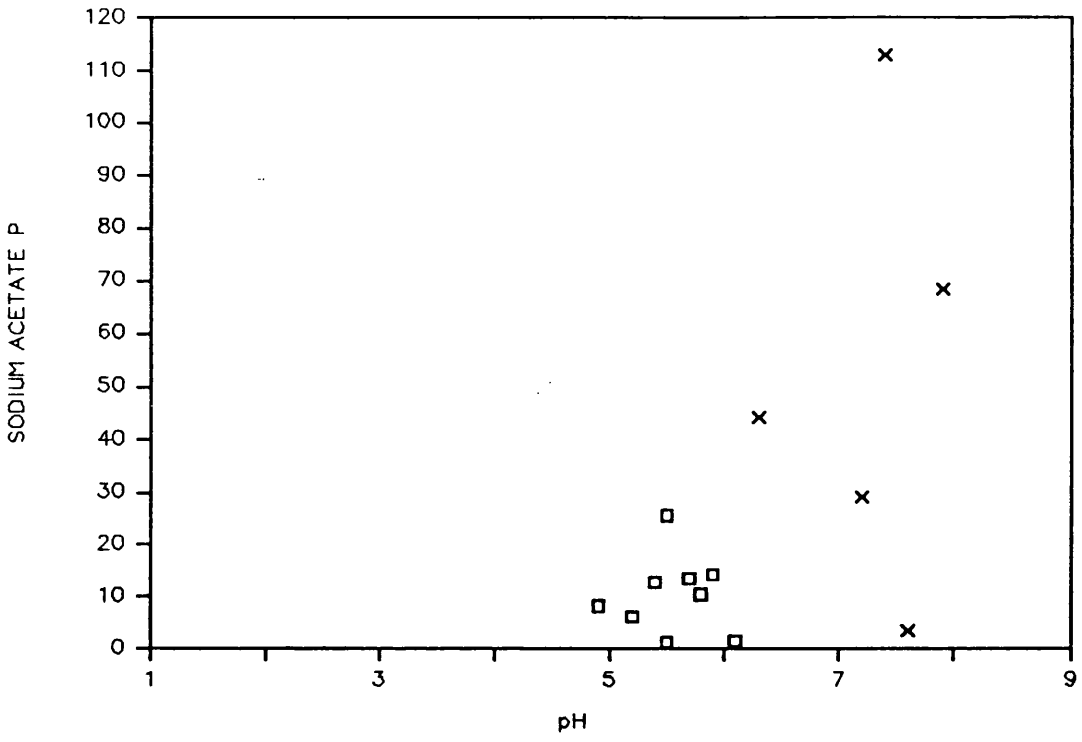


Fig 3.2 Scatter diagram showing the relationship between sodium acetate extractable P and pH of soils.

(□) Soils with pH < 6.2

(x) Soils with pH > 6.2

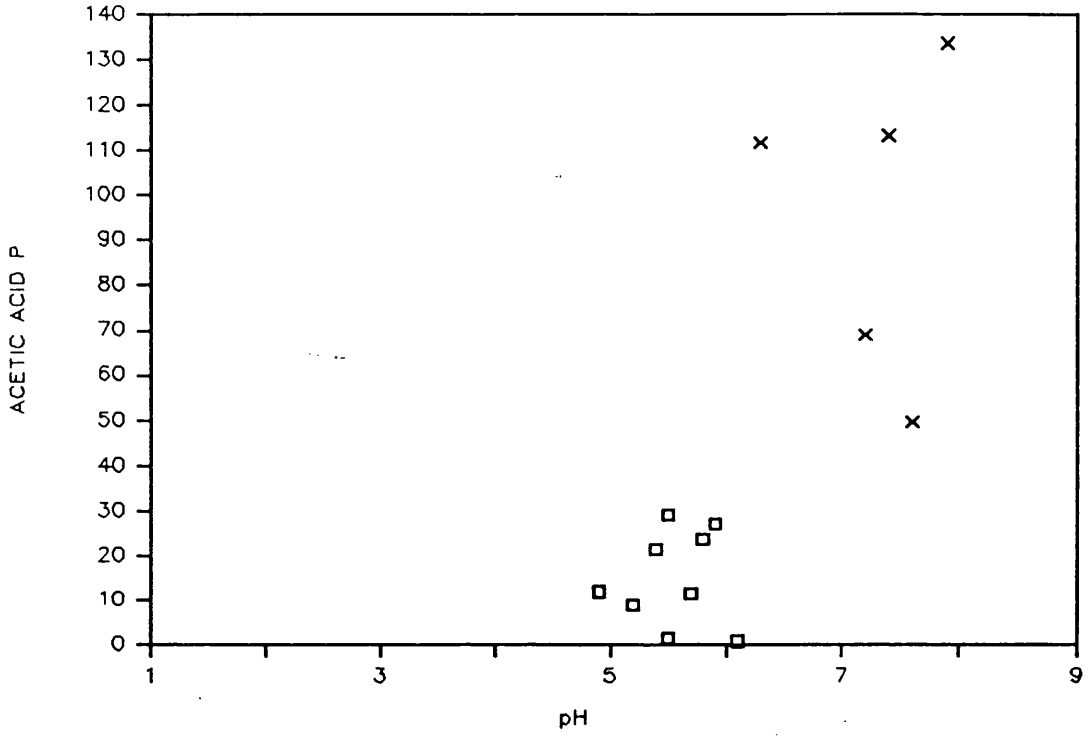


Fig 3.3 Scatter diagram showing the relationship between acetic acid extractable P and pH of soils.

(□) Soils with pH < 6.2

(x) Soils with pH > 6.2

Table 3.3 Correlation coefficients between soil phosphate using six extractants from 9 soils having pH 5.2 to 6.3

EXTRACTANTS	NH_4-acetate	Na-acetate	Acetic-acid	Na-bicarbonate	Bray-I
Na-acetate	0.97***				
Acetic acid	0.88**	0.86**			
$NaHCO_3$	0.89**	0.97***	0.83**		
Bray-I	0.73*	0.83**	0.75*	0.90***	
Anion exchange resin	0.97***	0.93***	0.80*	0.83**	0.61NS

NS = Non-significant at 5% * = Significant at 5%
** = Significant at 1% ***= Significant at 0.1%

Table 3.4 Correlation coefficients between soil phosphate using six extractants from 5 soils having pH from 6.3 to 7.9

EXTRACTANTS	NH_4-acetate	Na-acetate	Acetic-acid	Na-bicarbonate	Bray-I
Na-acetate	0.99***				
Acetic acid	0.82NS	0.76NS			
$NaHCO_3$	0.95*	0.98*	0.67NS		
Bray-I	-0.31NS	-0.29NS	-0.01NS	-0.16NS	
Anion exchange resin	0.52NS	0.54NS	0.62NS	0.621NS	0.636NS

NS = Non-significant at 5% * = Significant at 5%
** = Significant at 1% ***= Significant at 0.1%

extractants were decreased in alkaline soils by splitting (Table 3.4), except correlation between ammonium acetate and sodium acetate (Fig 3.4). In some cases the two highly alkaline soils, Alluvium and Swaffham Prior were out of the line (Fig 3.5 to 3.6), while in the other cases all the alkaline soils adopted separate trend of relationship (Fig 3.1 to 3.2).

3.3.3. Comparing the effectiveness of extractants

3.3.3.1 Acid ammonium fluoride (Bray-I reagent)

Though the highest amount of soil phosphate extracted in this study, was by this method, yet it removed a negligible amount of phosphate in alkaline soils. The relationship between pH and extractable Bray-I P was negatively significant at 1% level of probability ($r=-0.67^{**}$) in all 14 soils. A decrease in extractable Bray-I P with increase in pH has also been reported by Griffin (1971). In a study of extractability of phosphate from a range of calcareous soils, Smith et al. (1957) found that the addition of 0.03 M NH_4F to 0.025 N HCl greatly decreased the amount of phosphate extracted. The lower estimate of phosphate obtained by this method, in calcareous soils has been attributed to the rapid neutralization of the acid by $CaCO_3$ and deactivation of F^- ion by the calcium contained in these soils (Randall and Grava, 1971). Smillie and Syers (1972) reported formation of CaF_2 and consequent immobilization of P

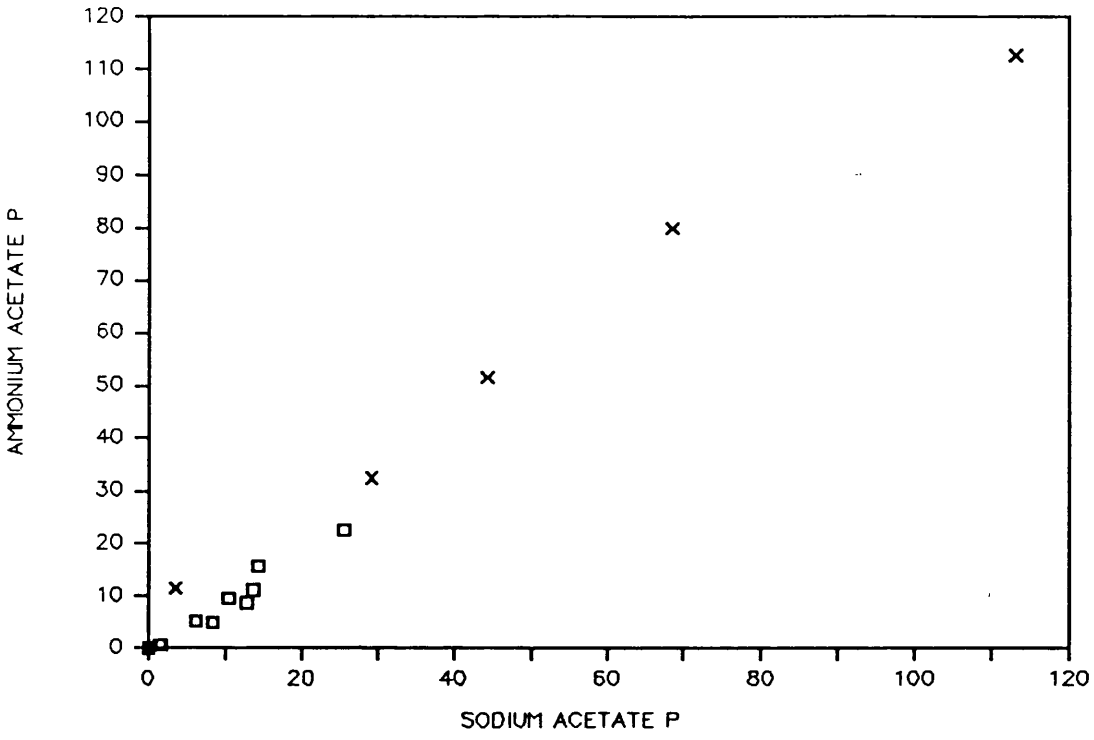


Fig.3.4. Scatter diagram showing the relationship of sodium acetate and ammonium acetate extractable P.

(□) Soils with pH < 6.2

(x) Soils with pH > 6.2

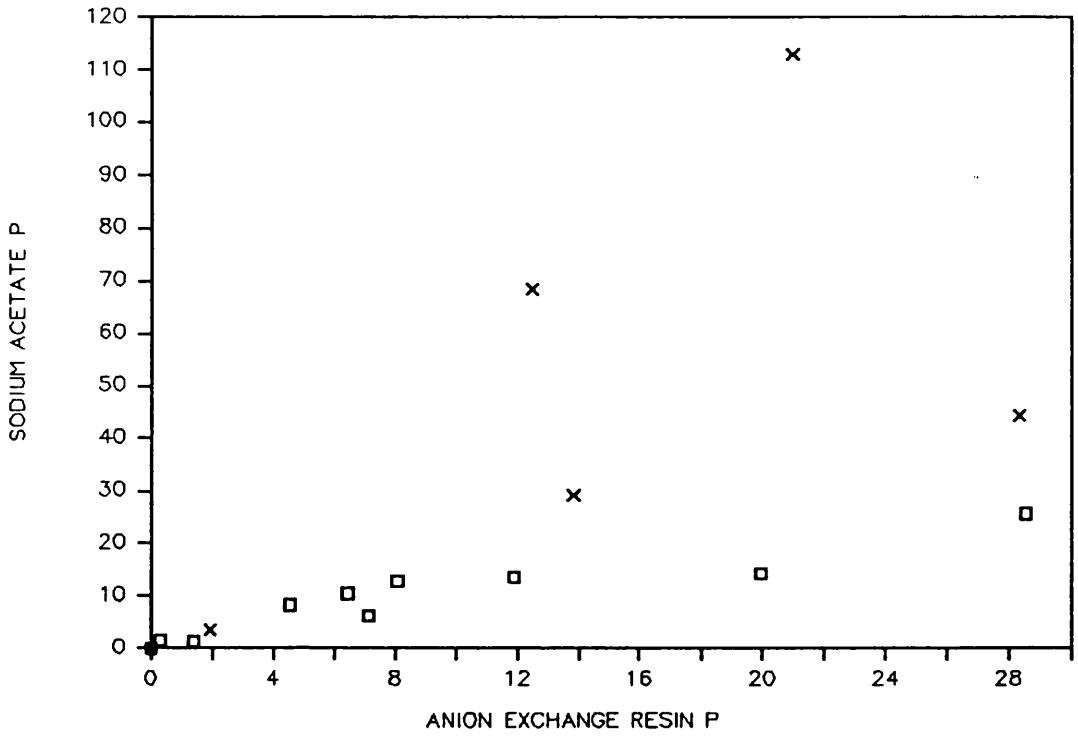


Fig 3.5 Scatter diagram showing the relationship between sodium acetate and anion exchage resin extractable P.

(□) Soils with pH < 6.2

(x) Soils with pH > 6.2

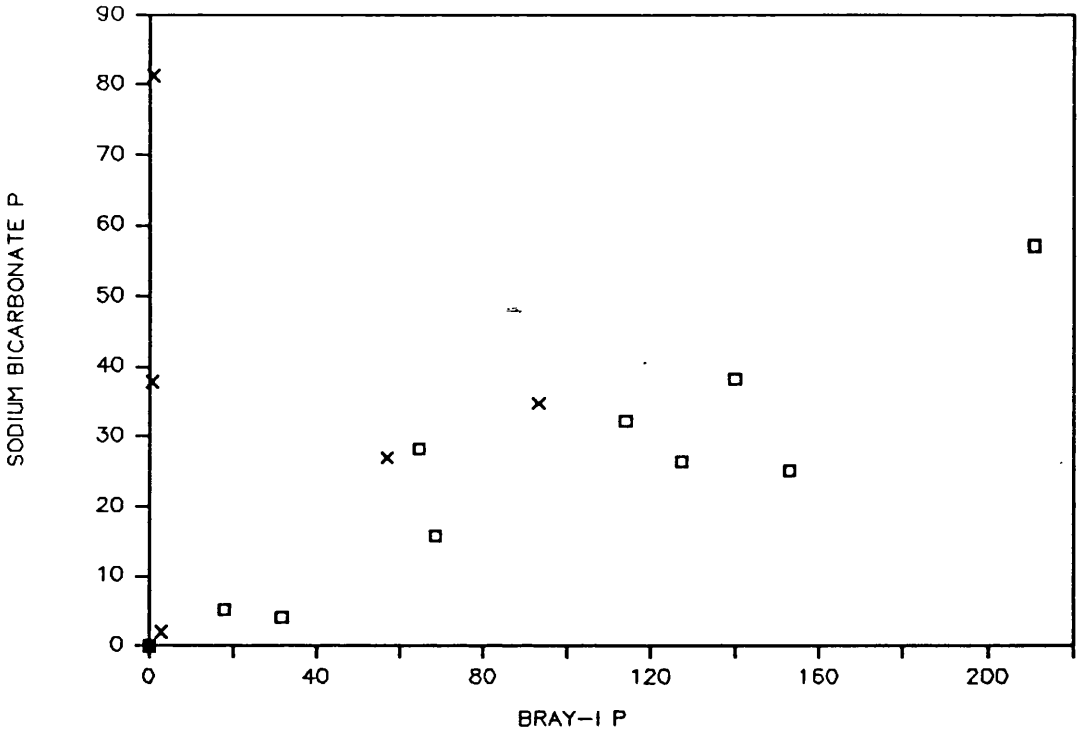


Fig 3.6 Scatter diagram showing the relationship between sodium bicarbonate and Bray-I extractable P.

(□) Soils with pH < 6.2

(x) Soils with pH > 6.2

during extraction of calcareous soils with the Bray P-I reagent. Many of the soils investigated by Smith et al. (1957) had a high P status and showed the repressive effect of fluoride. Yerokun (1987) reported that the amount of P extracted was the highest in Bray P1 test, as compared to Olsen, anion exchange resin and $CaCl_2$ methods, except in calcareous Charity clay soil. Hence Bray method was regarded as more effective in detecting the soil testing low in P. In this study relatively more phosphate was extracted in most of the soils, may be due to strong acid and fluoride content, by enabling it to remove all forms of phosphate (Radwan et al., 1985; Hons et al., 1990). Menon et al. (1989) concluded from their results that Bray-I solution extracted more P than other tests and resulted in an over estimation of plant available P.

The active fluoride ion coupled with strong acid has made this reagent capable of extensive attack on all forms of inorganic P (William and Knight, 1963). The fluoride ion can form soluble complexes with Aluminium in soils even when >90% total soil fluoride is adsorbed (Moore and Ritchie, 1988). The extractability of various phosphate fractions by this extractant was in the order: Al-P>Ca-P>adsorbed-P>Fe-P (Somani, 1989). Due to complexing of cations binding P feature (Kamprath and Watson, 1980) and strong electronegative nature of fluoride ion made it very effective in releasing

adsorbed P (Manikandan and Sastry, 1986). The method proposed by Bray and Kurtz (1945) has been regarded the most successful in areas of wide range of acidic to neutral soils (Yee and Broersma, 1987), acid soils (Olsen and Sommers, 1982; Patiram and Prasad, 1990), neutral and calcareous soils (Randall and Grava, 1971).

In the relationship studies of various soil characteristics with extractable Bray-I P, it revealed significant correlation of extractable P with pH, Al and Fe. But no significant correlation with LOI, organic matter and soil texture was observed.

3.3.3.2 0.5 M Acetic acid

The method ranked second in extracting the highest amount of phosphate from all 14 soils. Contrary to Bray-I method, it removed higher amount of PO_4-P from alkaline soils, presumably due to the larger proportion of calcium phosphate as suggested by John (1972) and Syers et al. (1972), while relatively lesser amounts of P was removed in acidic soils. These results agree with the findings of Van Lierop (1988). Mattingly and Pinkerton (1961) reported that acetic acid attacks P sources not available to plants. It showed very highly significant correlations with ammonium acetate buffer ($r = 0.90^{***}$) and sodium acetate buffer ($r = 0.85^{***}$). The method is widely used as index of available phosphate supply in soils, by the Advisory Service of Scotland (SAC, 1985).

Acetic acid extractable PO_4-P has very highly significant correlation with pH ($r = 0.78^{***}$) and Fe concentration ($r = -0.78^{***}$), while the correlations with other soil characteristics were found non significant.

3.3.3.3 0.5 M Sodium bicarbonate (Olsen's method)

The amount of phosphate extracted from all 14 soils by this method was in intermediate position. The PO_4-P content was statistically at par with ammonium acetate buffer and sodium acetate at 1% level of significance (Table 3.1). Though this method was originally developed by Olsen et al., (1954), for calcareous soils, but its performance in present study, was fairly good in acidic soils as well as in alkaline soils. It has been described the best method for estimating the available phosphate, not only for calcareous soils but also for acid soils (Fardeau et al., 1988). Olsen's sodium bicarbonate method is most commonly used in the UK. It is very satisfactory on soils at pH 6.0 and above but less satisfactory on acid soils (Archer, 1988). According to Olsen et al., there are two major mechanisms in $NaHCO_3$ extraction process:

(i) Calcium phosphates increase in solubility in $NaHCO_3$ as a result of the repression of Ca^{2+} activity (Common ion effect of CO_3^{2-} ions in the presence of solid phase $CaCO_3$); and (ii) The HCO_3^-, CO_3^{2-} and OH^- ions replace P ions on the surface of soil particles. They further explained that the main effect of $NaHCO_3$ in calcareous

soils is to decrease the Ca^{2+} activity which in turn increase the solubility of P. Whereas in acid and neutral soils the main effect is through ionic competition of HCO_3^-, CO_3^{2+} and OH^- ions for P adsorbed on the surface of particles. Aminuddin et al. (1987) reported that the bicarbonate ions in the buffered alkaline solution replace adsorbed P and is effective in extracting Al-P and to some extent Fe-P but not Ca-P.

No significant relationship of $NaHCO_3$ extractable P with any of the soil characteristics was observed in combined group of soils, acidic soils and alkaline soils except in case of fine sand fraction in alkaline soils ($r = 0.97^{***}$).

3.3.3.4 Sodium acetate buffer (Morgan's reagent)

The amount of PO_4-P extracted by this extractant was statistically comparable with sodium bicarbonate and ammonium acetate buffer (Table 3.1). This method showed very highly significant correlation with ammonium acetate ($r = 0.99^{***}$), 0.5M acetic acid ($r = 0.85^{***}$), $NaHCO_3$ ($r = 0.81^{***}$) and significant with anion exchange resin ($r = 0.55^{*}$) in all 14 soils under study. A significant correlation of this method with $NaHCO_3$ ($r = 0.711^{**}$) has also been reported by Gupta and Cornfield, (1963) from 15 calcareous soils. Whereas Reith et al. (1987) found this test very highly significantly correlated with acetic acid, bicarbonate, AER and lactate methods. Similar

results of high degree of correlation between the estimates of phosphate by $NaHCO_3$ and sodium acetate obtained by Kuo et al., (1988). In computation of different forms of the plant utilizable P, Somani (1989) found a linear relationship of Morgan's P with Al-P and adsorbed P.

The amount of PO_4-P extracted by this method has no significant correlation with all soil characteristics studied in all 14 soils except pH and Fe content, which have significant coefficients of correlation 0.61^* and -0.59^* respectively. In alkaline soils group, fine sand had very highly significant correlation and Fe content negatively significant correlation with extractable P. While no significant correlation of extractable P was observed in acidic soils, with any of the soil characteristics under study.

The amounts of phosphate extracted by Morgan's solution was lower than the amounts removed by other extracting solutions like Bray-I and Olsen's reagent, in acidic soils, but it removed relatively higher amounts of phosphate in alkaline soils. Peech and English (1944) considered this method better than other solution of strong acids or neutral salts, having certain desirable features.

3.3.3.5 Ammonium acetate buffer (pH 4.8)

The performance of this test was almost similar to Morgan's method. The extractable PO_4-P by this method did not significantly differ from ammonium acetate and sodium bicarbonate method, at 1% level of probability (Table 3.1). The coefficients of correlation of this method with sodium acetate, acetic acid, $NaHCO_3$ and anion exchange resin were found 0.99^{***}, 0.90^{***}, 0.75^{**} and 0.54^{*} respectively.

In the study of relationship of extractable PO_4-P with soil properties, it was observed that pH and Fe had highly significant correlation with extractable P by this method. Similar effect of pH on ammonium acetate extractable P has also been reported by Griffin (1971). In alkaline soils, like Morgan's method, very highly significant correlation of fine sand and negatively significant correlation of Fe content with extractable P were observed. However, in this case, negatively significant correlation of clay content with extractable P was also recorded.

3.3.3.6 Anion exchange resin

From perusal of the data in Table 3.1, this method extracted the lowest quantity of phosphorus. Anion exchange resin, presumably removed phosphate which is physically sorbed by soil material (Tiessen, 1984) and more loosely bound P (Sharpley, 1991). Though Enwezor

(1977) extracted the lowest value of phosphate by anion exchange resin, yet it was considered superior because it gave better correlation with percentage yield. By comparing the correlation coefficients for the six extractants, anion exchange resin has good correlation with ammonium acetate ($r = 0.55^{*}$) and sodium acetate ($r = 0.55^{*}$), better correlation with $NaHCO_3$ ($r = 0.73^{**}$) and non-significant correlation with Acetic acid and Bray-I methods in all the 14 soils. By splitting the soils into two groups based on soil pH, the correlation coefficients with all other methods improved in acidic soils except Bray-I. In alkaline soils the resin method showed non-significant correlation with all the extractants in this study. However Reith et al. (1987) compared the relationship of anion exchange resin with four other extractants and found it the best method. Although the extracting P by anion exchange resin is superior to the other methods for predicting the response to P it is more time consuming, difficult to carry out on large scale and only suitable for single measurement (Sibbesen, 1978; SAC, 1985).

Anion exchange resin extractable P did not show any significant relationship with any of the soil characteristics, in any of the soil groups.

3.4 Conclusions

Available soil phosphate determined by various chemical methods, has been widely used as the basis for fertilizer recommendations. The determination of available phosphorus of any group of soils by different methods, however, frequently, results in conclusions that differ in respect to the phosphorus status of the soils concerned. Many workers have studied the correlation between results for available phosphorus as determined by various methods. The literature does not show, however, any precise explanation of the differences in degree of correlation between different pairs of methods from the point of view of soil chemistry.

It is a well known fact that available soil phosphate determined by a chemical method includes all forms of inorganic phosphates, but mainly aluminium, iron and calcium phosphate. The total and relative amounts of phosphorus dissolved from the various forms of phosphates depend on the relative amounts of calcium, aluminium and iron phosphate in a soil, and relative solubilities of these phosphates in the extractants.

CHAPTER 4

PHOSPHATE ADSORPTION BY SOILS

4.1 Introduction

4.1.1 Phosphate adsorption

The adsorption of phosphate is a process in which phosphate ions in solution react with atoms on the surface of soil particles. This is an important property affecting both the fate of phosphatic fertilizer and the availability of phosphate to plants.

The P concentration in soil solution is very low compared to plant requirements, it is necessary to continuously replenish this pool to avoid P deficiency. The labile P fraction serves this purpose. The labile soil P consists of P weakly adsorbed on to the soil surfaces. This fraction is in equilibrium with solution P and is considered to be potentially available for plant use (Larsen et al., 1965). The concentration of P in this fraction could be 10^2 to 10^3 times greater than in solution (Mengel and Kirkby, 1982). Depending on time and soil P retention characteristics, the labile pool can become more stable and move into a non-labile pool (Barrow and Shaw, 1975). Non-labile P is strongly held on and within the clay lattices or is occluded by some other precipitation-recrystallization reaction. This fraction

reverts slowly into labile P, and is considered to be non-available to plants. When the soil P equilibrium is disturbed by adding fertilizer, reaction between fertilizer and soil takes place in two steps: a rapid step leads to the adsorption of P and a slow reaction converts P into a more firmly held form (Barrow, 1974,1978; Barrow et al., 1981).

The adsorption is commonly measured by shaking samples of the soil with standard phosphate solutions, measuring the change in phosphate concentration and calculating the phosphate adsorbed. The phosphate concentration in solution (Intensity) is measured and phosphate adsorbed (Quantity) is calculated. The information so gained is then summarized by plotting quantity against intensity. Such plots are known as Q/I plots. If this relationship is observed on a constant temperature, then the phenomenon is regarded as an adsorption isotherm (Prutton and Maron, 1953). The ratio between the adsorbed P quantity (Q) and soil solution P intensity (I) at an equilibrium concentration is regarded as the buffering capacity of a soil. The P supply to plants is governed by this property (Holford and Mattingly, 1976b). It is the indication of the ability of the soil to replace a unit change in soil solution P and maintain a productive solution concentration. The soil P buffering capacity may be the limiting factor in P uptake (Holford, 1976; Nair and Mengel, 1984). Phosphorus

buffering capacities are derived from adsorption isotherm plots (Holford, 1976; Parfitt, 1978; Olsen and Khasawneh, 1980).

A system has been used for predicting the amounts of phosphatic fertilizer required to bring soils to various levels of adequacy for crop production. An approach to this problem has been suggested by Beckwith (1964), who measured adsorbed phosphate at standard supernatant concentrations of P as an estimate of the phosphate needs of soils. Kurtz and Quirk (1965) evaluated the residual effects of phosphate fertilizers by using P adsorption isotherms. Phosphate adsorption isotherms have been used as basis for evaluating the phosphorus requirements of soils (Fox and Kamprath, 1970; Fox, 1981/82).

The adsorption isotherm is the most useful experimental procedure in the study of interaction of anions with oxides or soils and has been used by many workers to measure the adsorption capacity of soils (Olsen and Watanabe, 1957; Rennie and McKercher, 1959; Barrow, 1970,1978). The adsorption capacities of soils have been important criteria in soil classification (Rajan, 1973; Breeuwsma et al., 1986). The adsorption curves are an adequate basis for estimating P requirements across a diversity of soils and environments (Van der Zaag et al., 1979). Adsorption isotherms have advantages over conventional methods of soil testing, because the isotherms take into consideration both

intensity and capacity factors (Rajan, 1973; Tiarks, 1982).

Several factors apart from the nature of the soil used may affect the adsorption of phosphate. Barrow (1978) enumerated these as: the period and temperature of contact between soil and phosphorus solution; the method of shaking; the P solution : soil ratio; the identity and concentration of the supporting electrolyte used; the moisture content of the soil prior to treatment; and previous additions of phosphate or of other specifically adsorbed anions. The effects of these factors are described in chapter 1.

Investigations on phosphate adsorption have been centred on two major areas, namely the mechanism of phosphate adsorption and the identification of phosphate reactive sites. Understanding of the mechanism of phosphate adsorption is vital for improving efficiency in phosphate fertilizer use, while the identification of phosphate reactive sites is essential to evaluate the intensity of adsorption and understanding the various processes associated with the phenomenon. Compounds of iron and aluminium, particularly the oxide and hydroxide forms, and those of calcium, have been identified as the principal soil constituents active in phosphate adsorption (Holford and Mattingly, 1975; Parfitt, 1978; White, 1980).

4.1.2 Adsorption isotherms

Bache (1964), Muljadi et al. (1966) and Olsen and Khasawneh (1980) revealed that the isotherm from a plot of phosphate retained against equilibrium concentration could be divided into three regions corresponding to three distinct stages in the soil-phosphate interaction: (a) The first region corresponds to low phosphate addition resulting in practically complete adsorption or a negligible fraction of the added phosphate remaining in equilibrium solution. The adsorption isotherm rises steeply and remains close to the Y-axis. (b) The second region is the strongly curved portion of the isotherm which is convex to Y-axis. Bache (1964) showed that adsorption in this region varies logarithmically with the equilibrium phosphate concentration. (c) The third portion of the isotherm approaches linearity and occurs at medium to high phosphate concentrations. Here the adsorption varies linearly with the amount of phosphorus in equilibrium solution. At high level of this region, the slope of line is small and the isotherm, for most soils, tends to become more or less parallel to the X-axis. Freeman and Rowell (1981) have also described the reaction isotherms of phosphate with calcite surfaces in three stages.

4.1.3 Equations to describe soil-phosphate interaction

Several models have been used to describe the relationship between the amounts of adsorbed and dissolved species at a given temperature. The most popular equations for describing P adsorption by soils have been the Freundlich equation and the Langmuir equation.

The Freundlich equation was the first model to be used in describing phosphate retention (Russell and Prescott, 1916). Before the introduction of the Langmuir equation by Olsen and Watanabe (1957), the Freundlich equation was commonly used for describing phosphate adsorption data (Barrow, 1978). Bohn et al. (1985) advocated that the adsorption data from dilute solutions could be fitted to Freundlich equation in the following form:

$$X = KC^{1/n}$$ Where

K and n are empirical constants, C = Equilibrium concentration of adsorbate in mg/l and X = mg of adsorbed species/kg of soil.

The equation was originally empirical, without theoretical physico-chemical foundation, and no significance can be attached to the coefficients (Olsen and Watanabe, 1957; Holford, 1982). It implies that the

energy of adsorption decreases exponentially as the fraction of covered surface increases (amount of adsorption). The Freundlich equation can be derived theoretically by assuming that the decrease in energy of adsorption with increasing surface coverage is due to surface heterogenity. The Freundlich equation is normally used in its logarithmic form:

$$\text{Log } X = 1/n \text{ Log } C + \text{Log } K$$

A plot log X against log C should give a straight line. Though it is the oldest adsorption equation in the literature on phosphate adsorption, it has been shown to give better fits to adsorption data than the others (Gunary, 1970; Fitter and Sutton, 1975) especially in many soils over limited concentration ranges (Barrow and Shaw, 1975). Bache and Williams (1971) and Fitter and Sutton (1975) reported that plots of log adsorption versus log concentration gave gentle curves rather than the straight lines required by Freundlich equation. Barrow (1978) described two aspects relevant to this equation: one is that a logarithmic plot involves the transformation of the data in which values of low concentration are given a high weighting; the second aspect is the difficulty of taking adequate account of the phosphate already present in the soil and taking part in the equilibration. This problem is common to all equations. However Fitter and Sutton (1975), Barrow (1978) and Torrent (1987) have shown that the plot of

adsorption data according to the Freundlich equation gives straight lines if correction is made for the phosphate already adsorbed on the surface.

The Freundlich relationship has been used only for the theoretical treatment of phosphate adsorption. It has not been possible to compare quantitatively adsorption data for soils obtained from plots of the Freundlich equation. However some workers suggested that intercept and slope of a linear Freundlich plot could be used to compare phosphate adsorption in soils (Kuo and Lotse, 1974; Holford, 1982). The Freundlich equation has also the limitation that it does not predict a maximum adsorption capacity. Despite its limitation, the Freundlich equation was a better fit to phosphate adsorption isotherms in most soils than the more widely used Langmuir equation (Fitter and Sutton, 1975) and as good a fit as the more complex 'two-surface' Langmuir equation (Barrow, 1978; Sibbesen, 1981).

The Langmuir equation proposed by Langmuir (1918) for the adsorption of gases on to clean solid surfaces, was first used by Olsen and Watanabe (1957), to describe phosphate adsorption in soils. In its linear form the Langmuir equation can be written as:

$$C/X = 1/Kb + C/b$$

Where C = equilibrium concentration of phosphorus in solution ($\mu g\ P\ cm^{-3}$)

X = mg phosphate adsorbed/kg soil

b = adsorption maximum (μg P /g soil)

K = a constant related to bonding energy of soil for phosphorus(cm^3 μg^{-1}).

A plot of C/X against C should give a straight line, from which the adsorption maximum b = 1/slope and the constant K, related to energy of adsorption (K = slope/intercept) can readily be calculated.

Adamson (1960) lists three principal postulates of the Langmuir isotherm: (i) the energy of adsorption is constant, which implies uniform sites and no interaction between adsorbed molecules for gases, but for soil ions were adopted; (ii) adsorption is on localized sites, which implies no transitional motion of adsorbed ions in the plane of the surface; (iii) the maximum adsorption possible corresponds to a complete monoionic layer. Larsen (1967) reported that all of these postulates will hold for P in soil.

The use of the Langmuir equation appears satisfactory because its derivation is acceptable on theoretical grounds and it contains parameters, which have physico-chemical significance (Olsen and Watanabe, 1957; Holford et al., 1974), representing the extensive (adsorption capacity) and intensive (affinity) properties of the adsorbent for the adsorbate (Holford, 1982). However, deviations from the expected linearity have been reported at high phosphate additions (Olsen and Watanabe, 1957; Ryden et al., 1977b).

The Langmuir isotherm can often be used to give a measure of the energy by which phosphorus is bonded to the solid and an adsorption maximum. Based on this maximum, calculation of the degree of phosphorus saturation can be made which has been shown to be related to plant uptake of soil phosphorus (Gunary and Sutton, 1967; Holford and Mattingly, 1976b). Holford (1982) reported that the Langmuir equation gave a more useful estimate of affinity (bonding energy) and sorptivity (adsorption maximum) over a concentration range of 0-5 µg phosphorus cm^{-3}. These adsorption parameters can be utilized in differentiating adsorption properties between soils and characterizing the availability of labile phosphate in soils. The sorptivity parameter is an excellent index of the potential immobility or unavailability of labile phosphate in soils (Holford, 1979).

Larsen (1967) and Harter and Baker (1977) reported that the Langmuir plots are most commonly curvilinear. This problem has been resolved by assuming that theory is obeyed only at low equilibrium concentration (Olsen and Watanabe, 1957; Rennie and McKercher, 1959), by resolving the curve into two straight lines and equating to two adsorption mechanisms (Harter, 1968; Syers et al., 1973; Holford et al., 1974). Gunary (1970) obtained better fits of the Langmuir equation by adding a square root term. Rajan and Fox (1975) and Ryden and Syers (1975) have

used the two term Langmuir relationship to obtain improved understanding of the soil-phosphate interaction. Use of the two-term Langmuir equation has been considered appropriate for phosphate adsorption studies since phosphorus is retained in soils by surfaces with differing affinities for phosphate. The 'two-surface' Langmuir equation gives meaningful estimates of phosphate adsorption capacity (Holford et al., 1974), which is greatly underestimated by the simple Langmuir equation (Holford, 1982).

In all the models in use the only parameters considered are the equilibrium concentration ,C, and the retained phosphate ,X, either in the original form or in modified form by taking into account the phosphate already adsorbed as suggested by Olsen and Watanabe (1957), Bache and Williams (1971), Fitter and Sutton (1975) Mendoza et al. (1990).

4.2 Materials and methods

The adsorption study was carried out on 14 soils according to the method described in Section 2.8.2.1.

1 g air dried soil was weighed into a series of 4 oz glass bottles, and 50 cm^3 of solutions containing 0.5, 1.0, 5.0, 10.0, 20.0, 30.0, 40.0, 50.0, 70.0 and 100.0 µg P cm^{-3} were added to these bottles with a 50 cm^3 pipette. All phosphorus solutions were made in 500 cm^3 volumetric flasks from either 100 µg P cm^{-3} or 1000 µg P cm^{-3} stock

solutions of oven-dried KH_2PO_4 and the volume was made up with deionized water. The bottles were capped tightly. The soil and phosphorus solution in bottles were equilibrated by shaking on an end over end shaker for 24 hours. After filtering the soil suspension through Whatman No.2 filter paper, the clear filtrate was analysed for phosphorus by the method described in Section 2.6.2.2, using the Technicon Autoanalyzer. The concentration of the original phosphorus solution was also measured. The difference between the amount of phosphorus in solution after shaking and the amount initially present was calculated as the amount of phosphorus adsorbed by the soil in μg P g^{-1}.

4.3 Results and discussion

4.3.1 Phosphate adsorption isotherm

The graphical representation of adsorption isotherms of the fourteen soils used in this study was obtained by plotting the amount of phosphorus adsorbed by the soil against the equilibrium concentration of P in solution. These adsorption curves are compared in a series of figures 4.1 to 4.5 on a uniform scale. It is evident from these isotherms that each soil exhibited different adsorption characteristics.

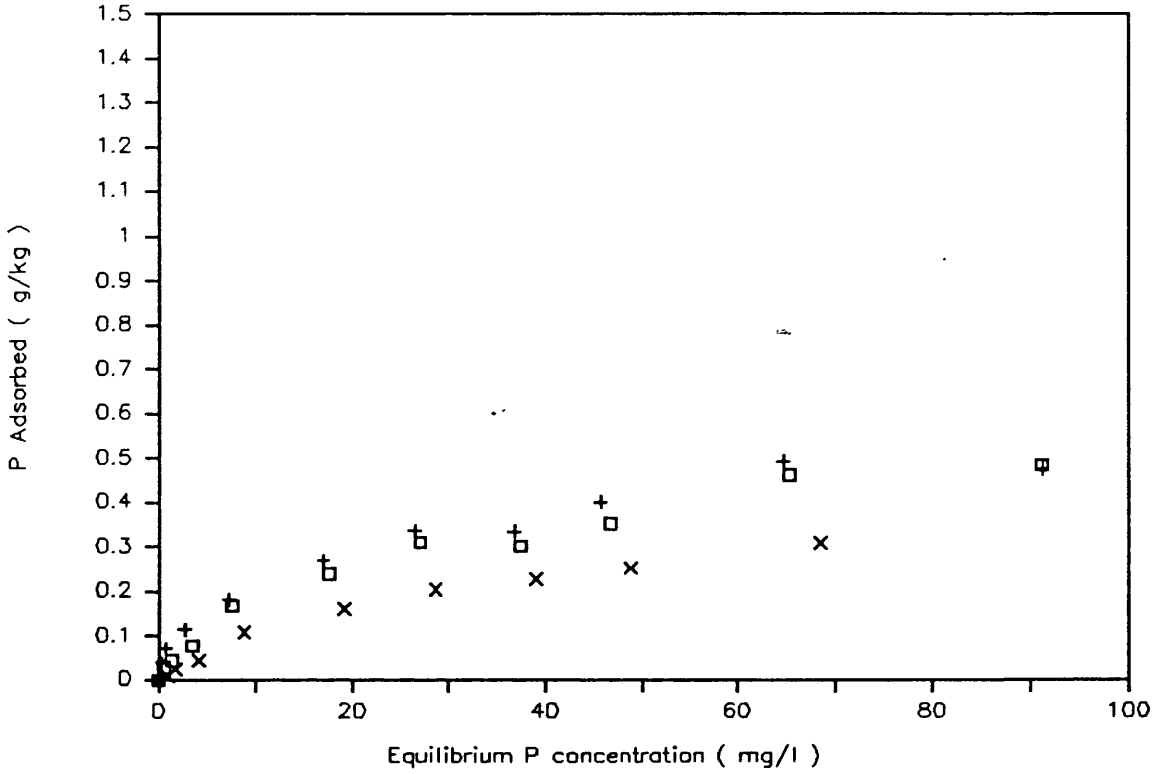


Fig. 4.1 Adsorption Isotherms for Dreghorn No.1A, Dreghorn No.1B, and Caprington A soils.

(□) Dreghorn No.1A

(+) Dreghorn No.1B

(x) Caprington A

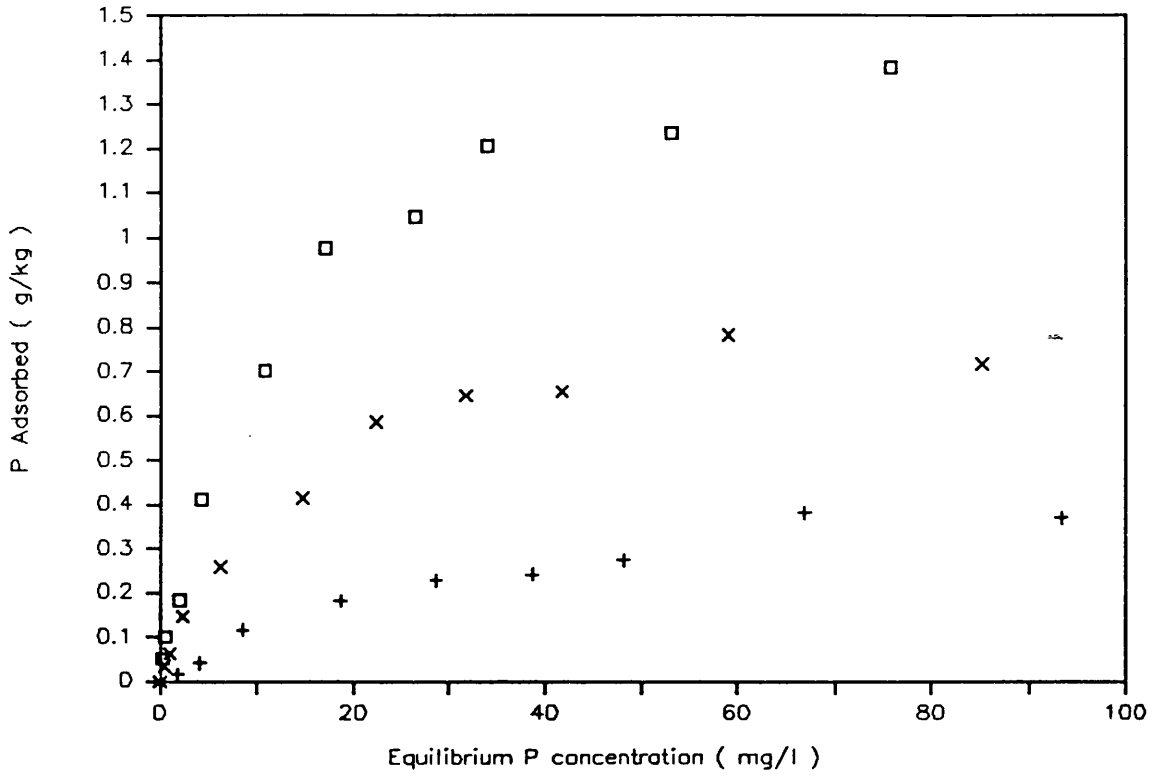


Fig. 4.2 Adsorption Isotherms for Dunlop A, Bargour A and Peat (Blackstoun) soils.

(□) Dunlop A

(+) Bargour A

(x) Peat (Blackstoun)

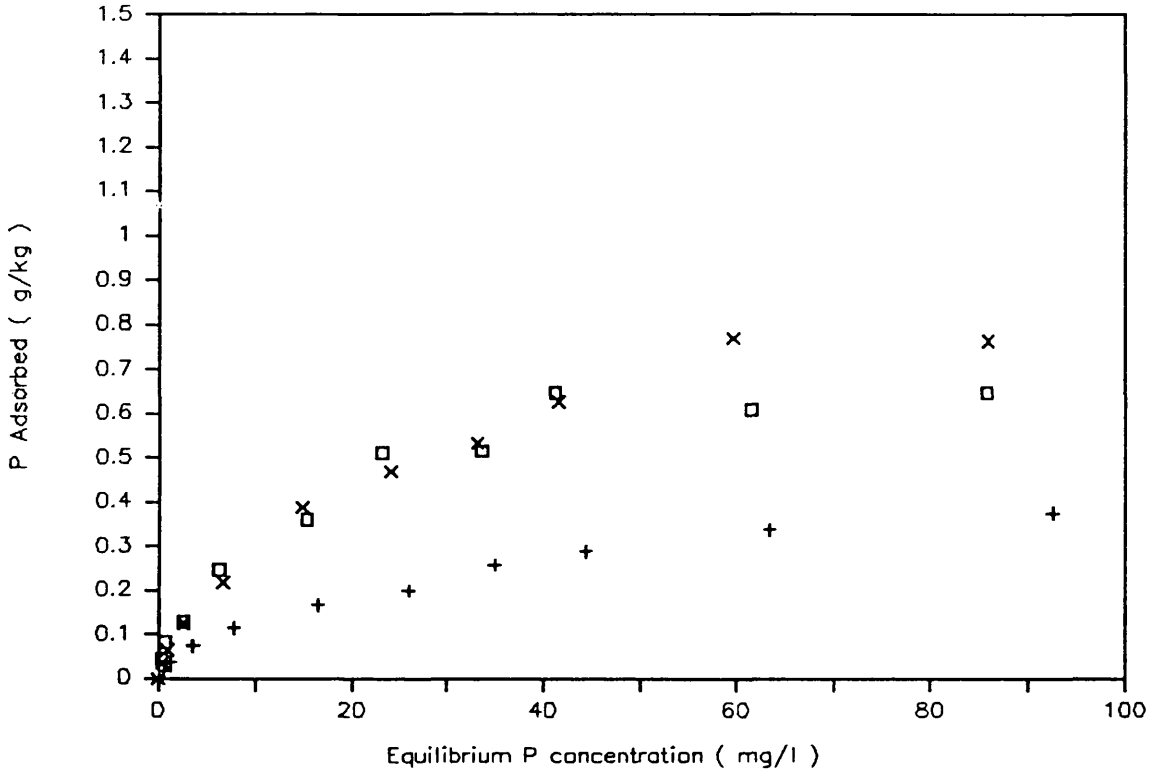


Fig. 4.3 Adsorption Isotherms for Dreghorn No.2A, Dreghorn No.2B, and Darvel A soils.

(□) Dreghorn No.2B

(+) Dreghorn No.2A

(x) Darvel A

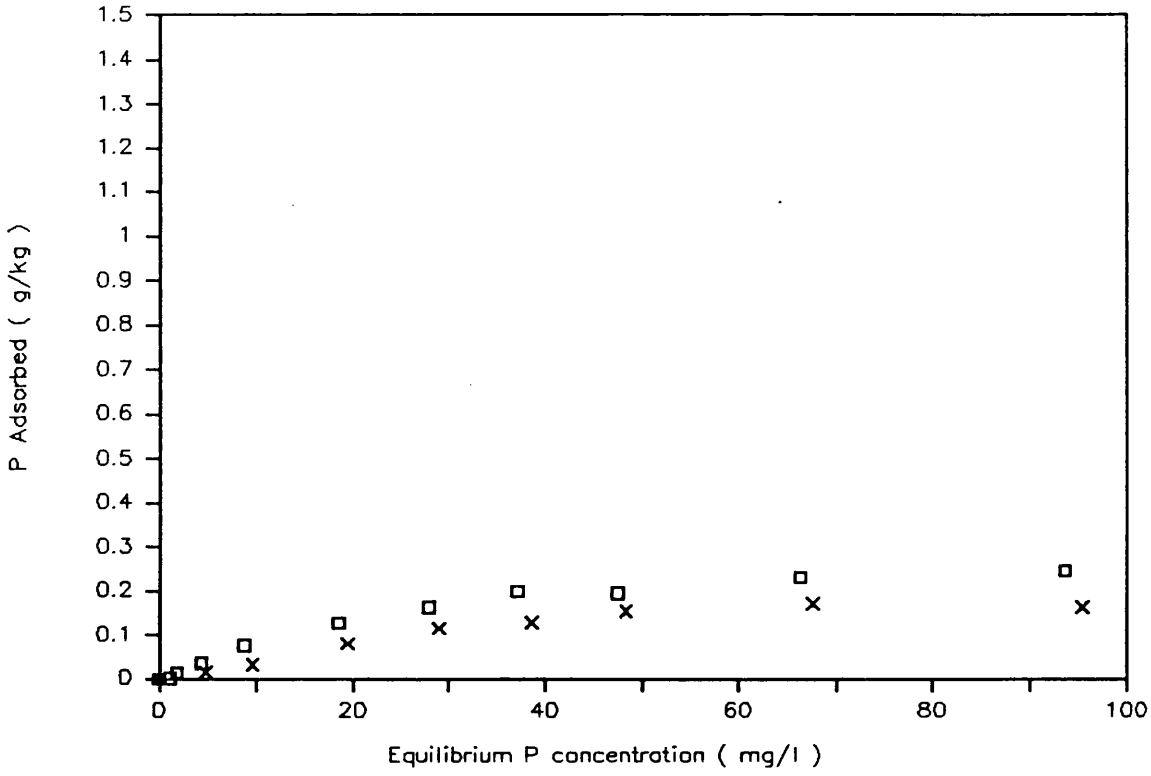


Fig. 4.4 Adsorption Isotherms for Swaffham Prior A and Alluvium soils.

(□) Swaffham Prior A

(x) Alluvium

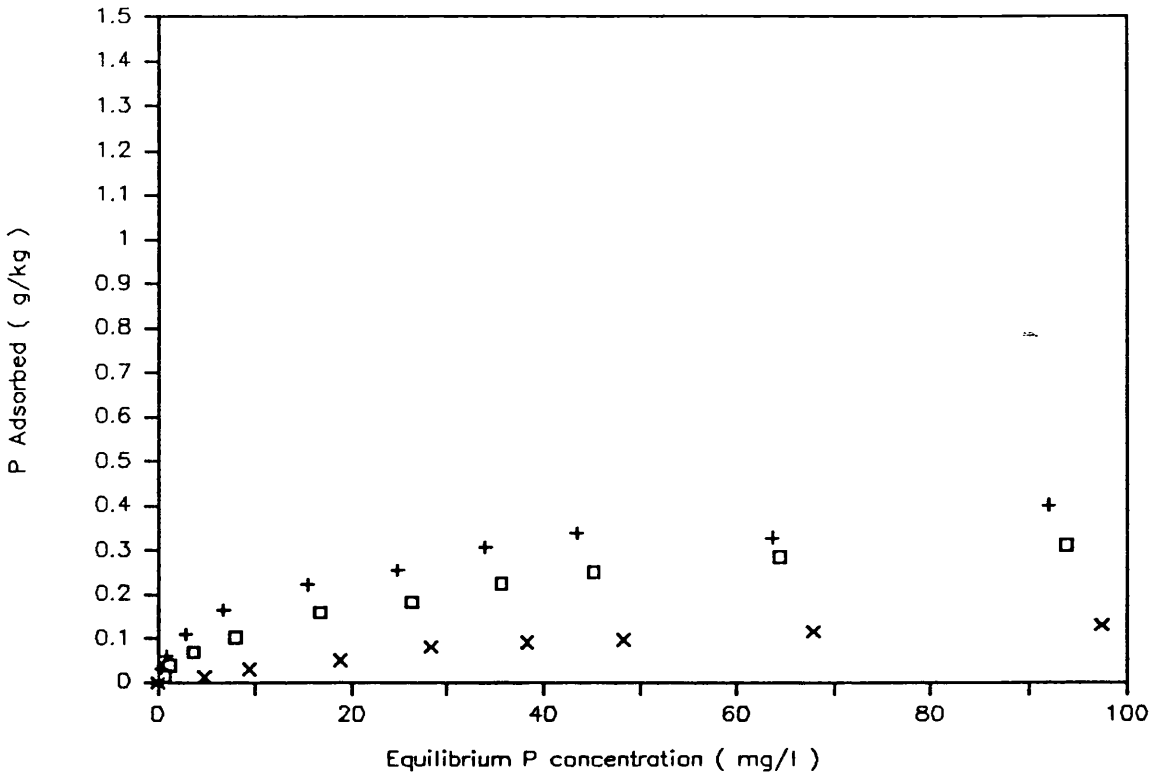


Fig. 4.5 Adsorption Isotherms for Midelney (Arable) A, Midelney (Arable) B and Midelney (Grass) A soils.

(□) Midelney (Arable) A

(+) Midelney (Arable) B

(x) Midelney (Grass) A

The soils can be arranged in the following descending order on the basis of the maximum observed values of P adsorbed:

Table 4.1 P adsorbed at the highest point of the isotherms of soils

Name of soil	Maximum P adsorbed (µg/g)
Dunlop	1400
Darvel	770
Peat (Blackstoun)	730
Dreghorn No.2 B	670
Dreghorn No.1 B	510
Dreghorn No.1 A	500
Midelney (arable) B	420
Bargour A	375
Caprington A	370
Dreghorn No.2 A	360
Midelney (arable) A	340
Alluvium A	170
Midelney (Grass) A	140
Swaffham Prior A	140

The P adsorption behaviour in the soils may have varied due to various reasons, such as their history of P fertilization, the pH value, soil texture and organic matter content. In some cases the subsurface soils showed relatively more P adsorption than surface soils: for example in Dreghorn No.2. An increase in P adsorption with depth could indicate that organic matter plays a role in reducing P adsorption in the upper soil horizons. Le Mare (1981) has given the same reason for more adsorption in subsurface samples, that most of the adsorption sites in the surface soils are occupied by organic anions. It has also been reported that arable

soils adsorb less P than their undisturbed counterparts, presumably because the cumulative amount of phosphatic fertilizer added to these soils has reduced their phosphate adsorption capacities (Toreu et al., 1988). Due to intensive arable production, some soils used in this study showed less P adsorption, for example, Midelney (Grass and Arable) and Peat (Blackstoun) soils. The Dunlop A being an uncultivated soil, on the other hand, showed the highest P adsorption. It has also been observed that in some soils the texture has become the basis of variation in P adsorption behaviour. Fine textured soils adsorb relatively more P than coarse textured soils, it may be either due to larger surface area or due to stronger reactive sites. Lower affinity for P by sandy soils has been indicated by Sibbesen (1981).

Though some workers have suggested these factors affect phosphate adsorption in soils, the data obtained in the present study give no clear indication of such influences.

Though some workers have divided the adsorption isotherm into three portions. It is very difficult in the present study to divide the isotherms into sections. In most of the soils the first portion of the curve is negligibly small and rises up just to 2 to 3 μg P cm^{-3} equilibrium concentration. In Dunlop A soil this portion of the curve rose up to 5 μg P cm^{-3} equilibrium

concentration (Fig. 4.2). The flexpoint was not obvious in certain soils where the number of sites for P adsorption was small and the sites were already occupied by residual P, as in Midelney surface soils (Fig. 4.5). At high levels of equilibrium concentration of P, the slope of the line is small and the isotherm tends to become parallel to the x-axis but it may never achieve this because a well-defined maximum is not obtained (Bache and Williams, 1971; Palacios Fernandez and Us'Yarov, 1990). For example in Bargour A and Peat (Blackstoun) soils, the adsorption isotherms approached a plateau at 80-100 μg P cm^{-3} equilibrium concentration (Fig. 4.2).

4.3.2 Fitting the equations to the data

The main objective of applying equations to phosphate adsorption curves is to summarize the adsorption properties of a soil using few parameters, to understand the nature of the adsorption process and to get a value for adsorption maximum. To achieve this end, the data obtained from the P adsorption experiments were fitted to the Langmuir and Freundlich equations. The Langmuir and Freundlich adsorption plots obtained from the experimental soils are illustrated in figures 4.6. to 4.19.

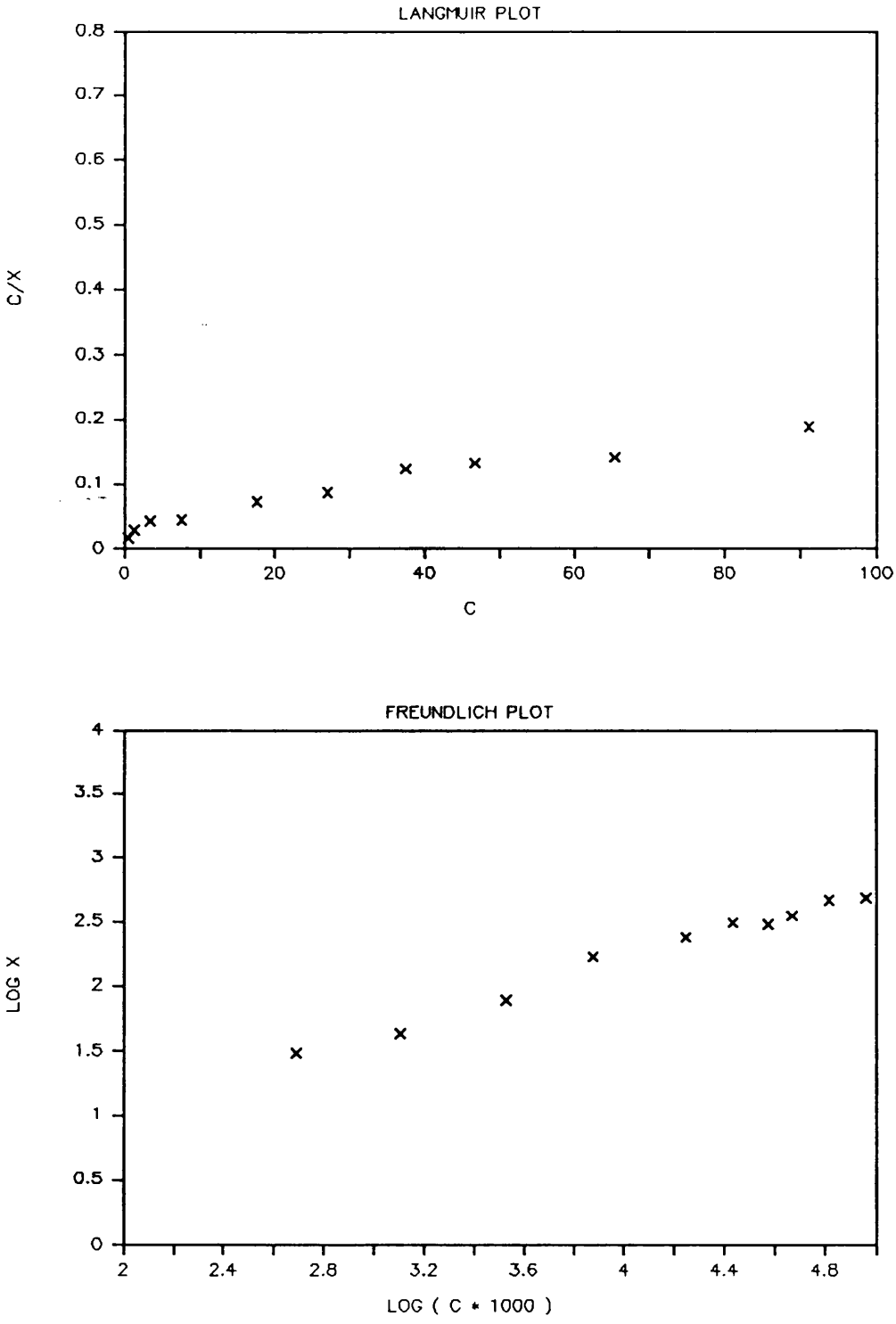


Fig. 4.6 Langmuir and Freundlich isotherms for the Dreghorn No.1 A soil.

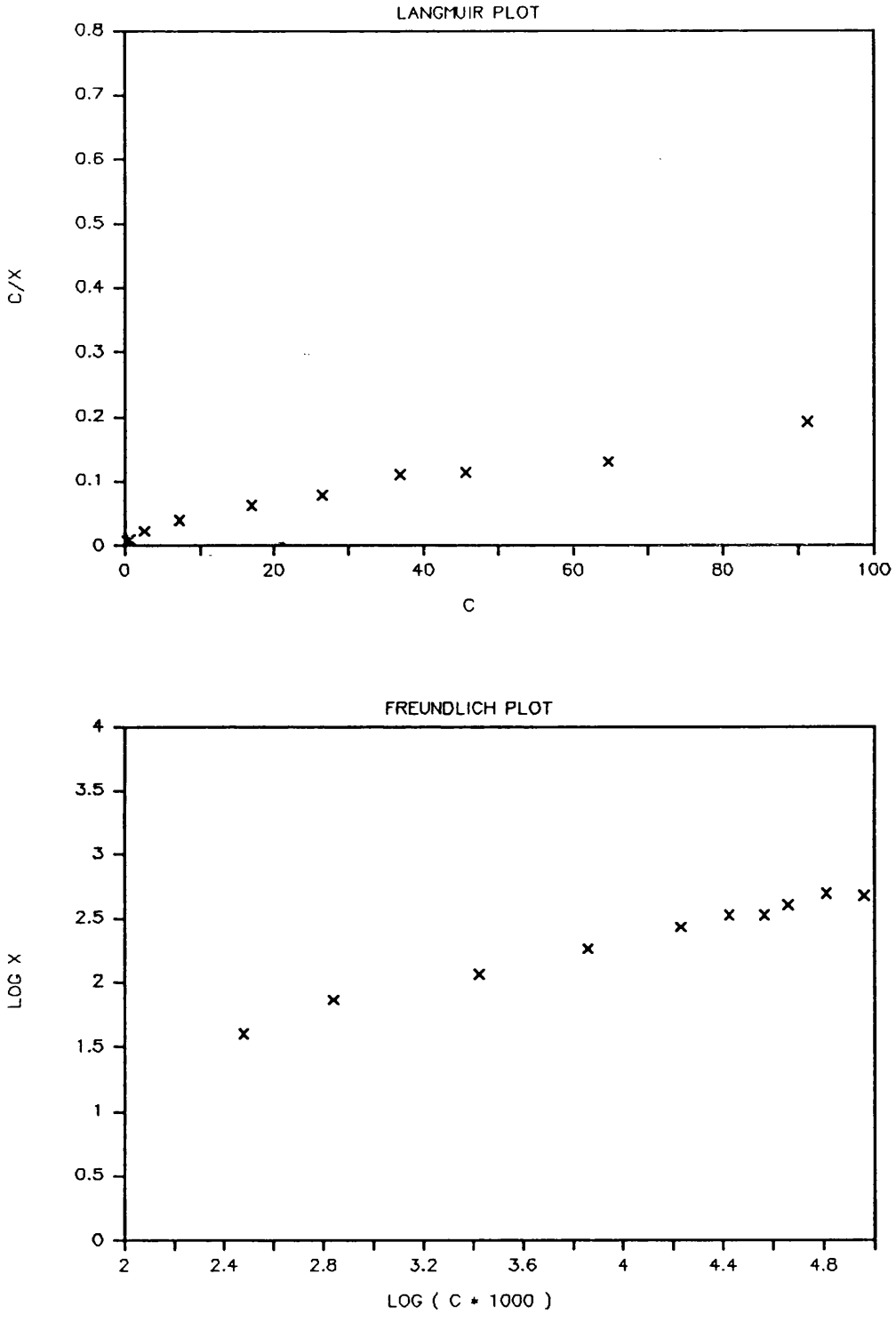


Fig. 4.7 Langmuir and Freundlich isotherms for the Dreghorn No.1 B soil.

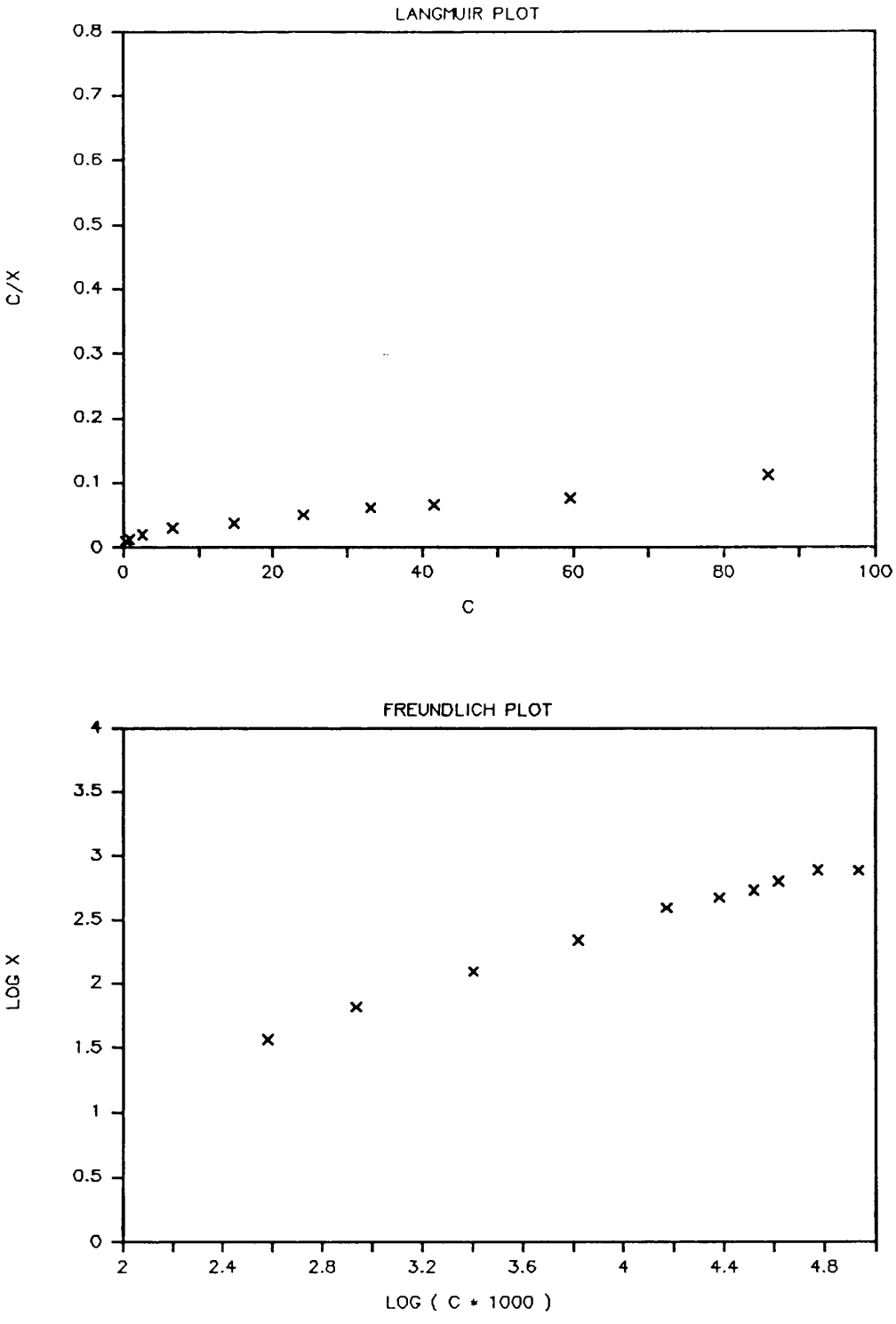


Fig. 4.8 Langmuir and Freundlich isotherms for the Darvel A soil.

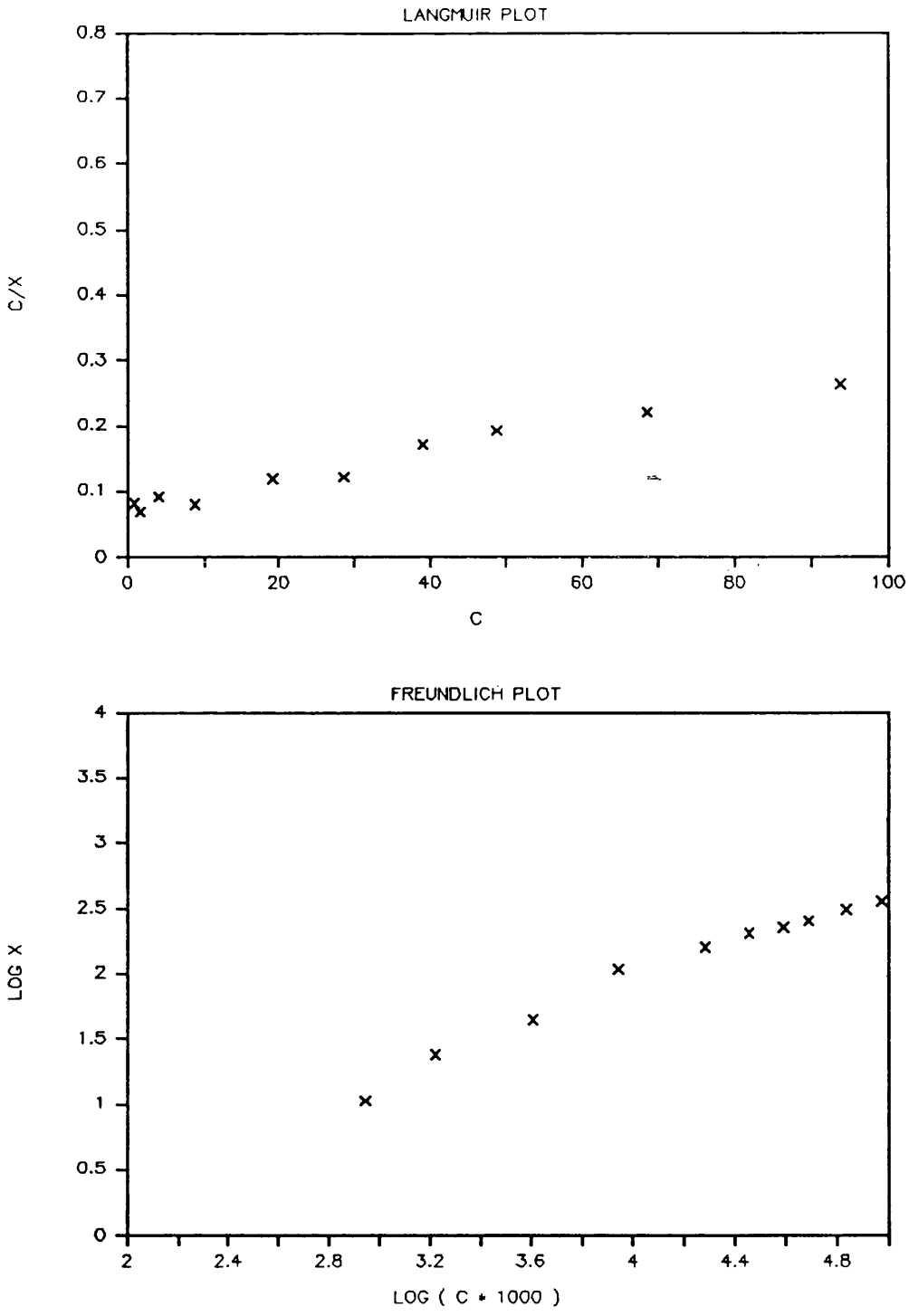


Fig. 4.9 Langmuir and Freundlich isotherms for the Caprington A soil.

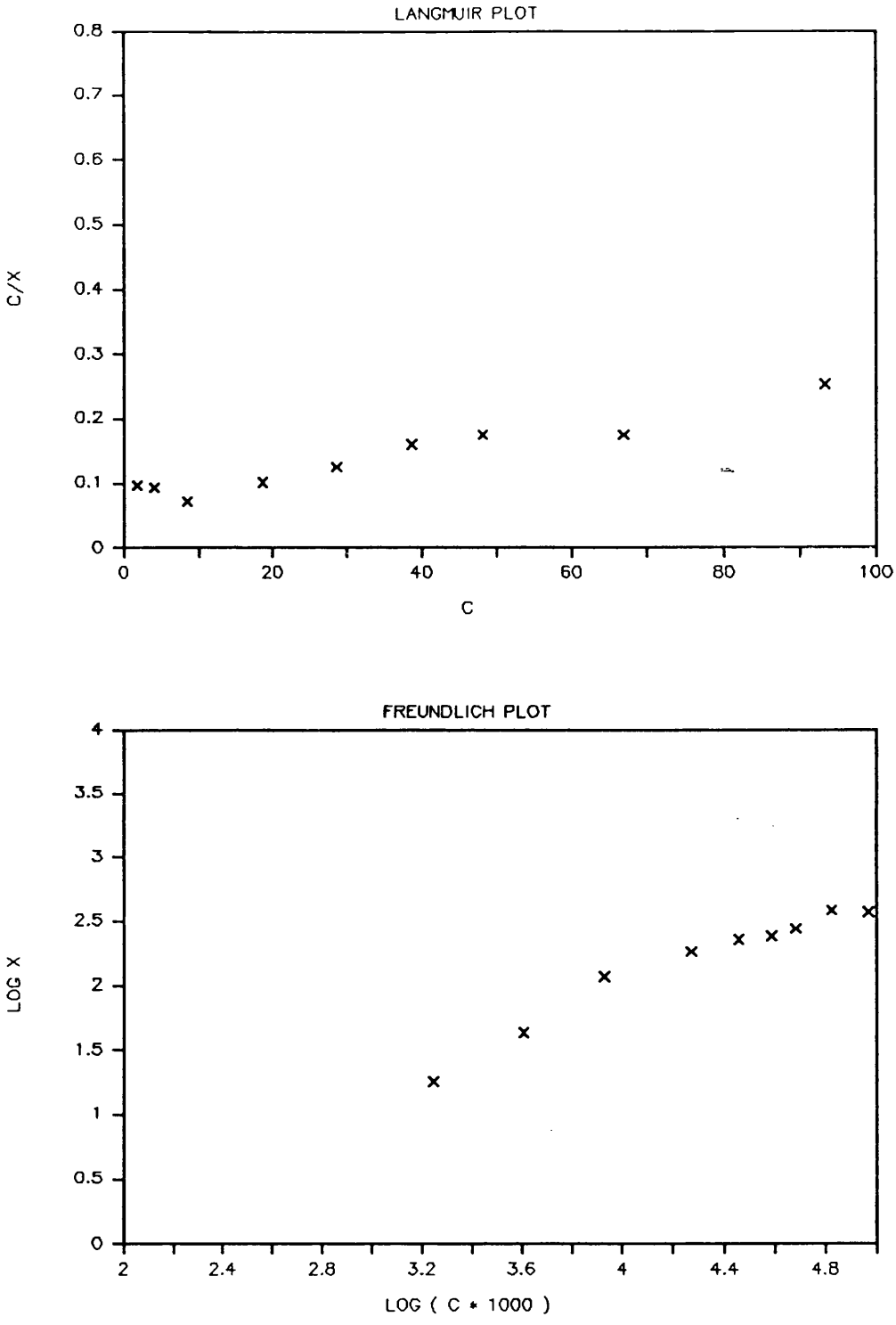
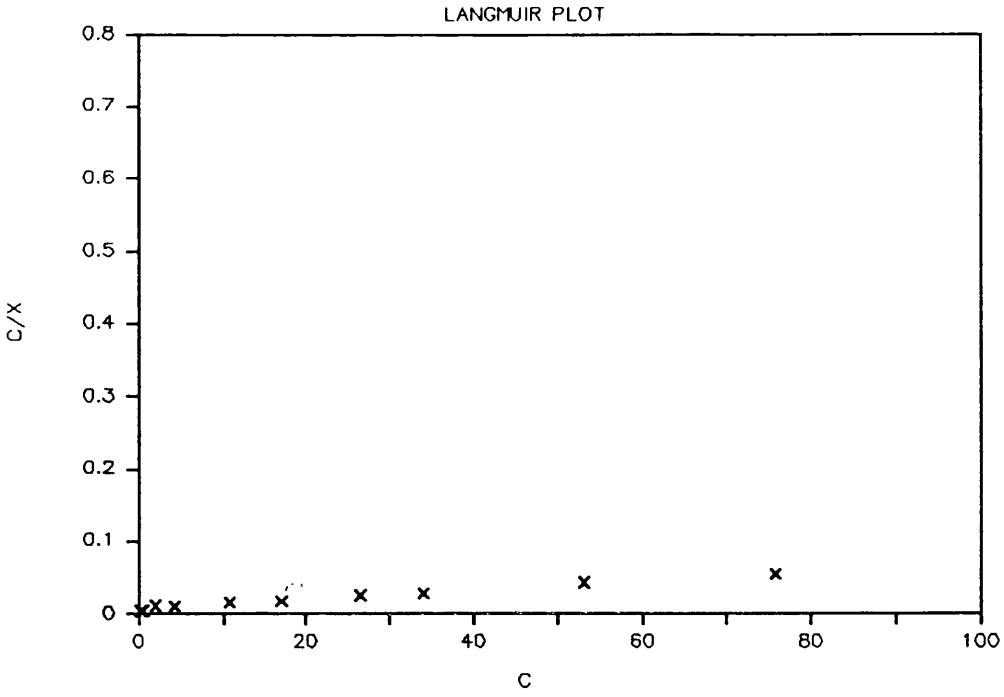


Fig. 4.10 Langmuir and Freundlich isotherms for the Bargour A soil.



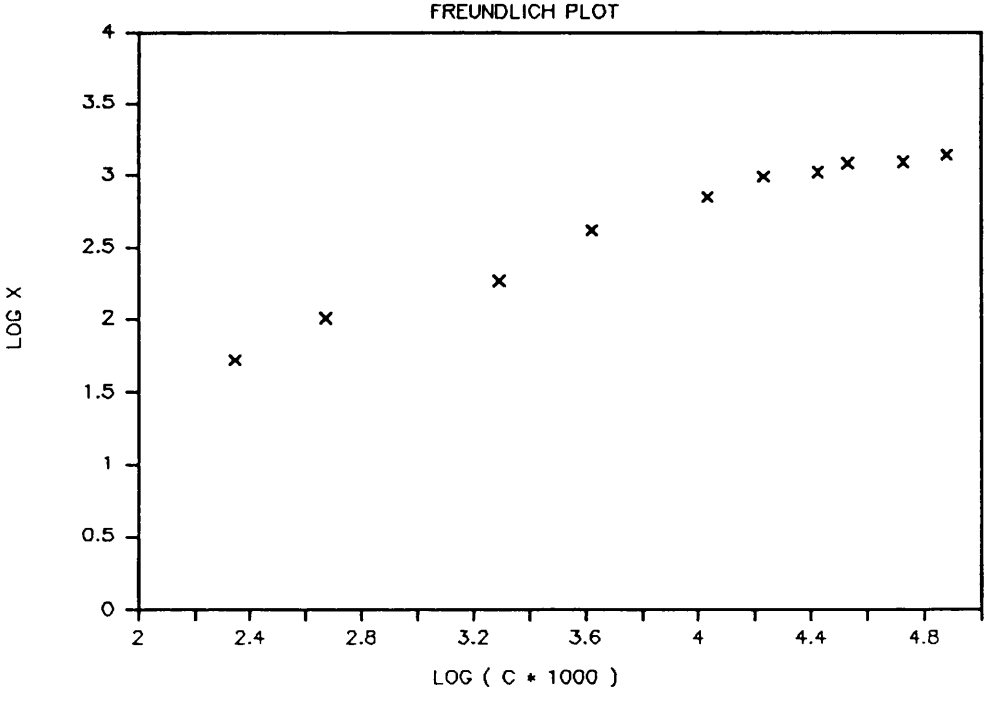


Fig. 4.11 Langmuir and Freundlich isotherms for the Dunlop A soil.

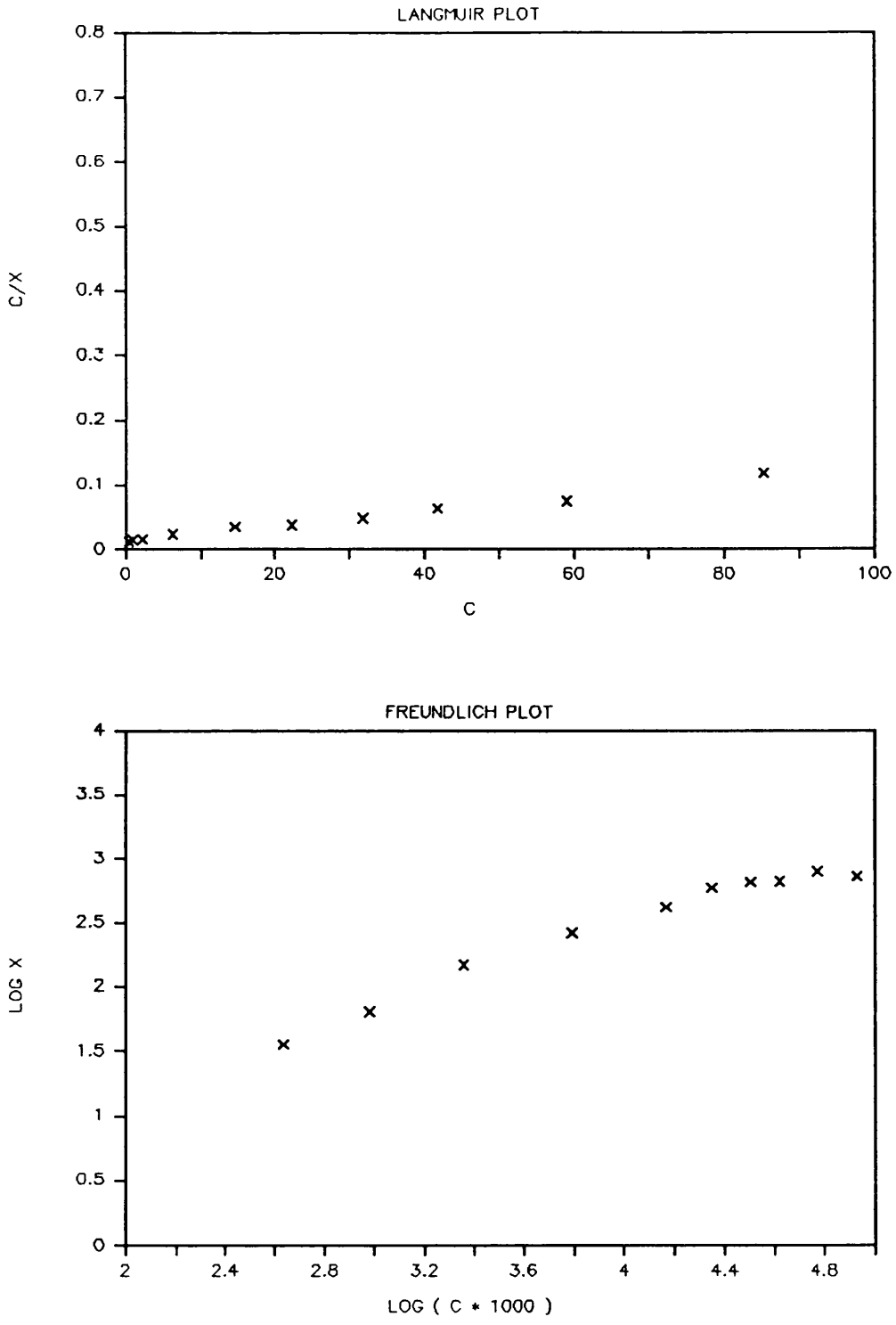


Fig. 4.12 Langmuir and Freundlich isotherms for the Peat (Blackstoun) soil.

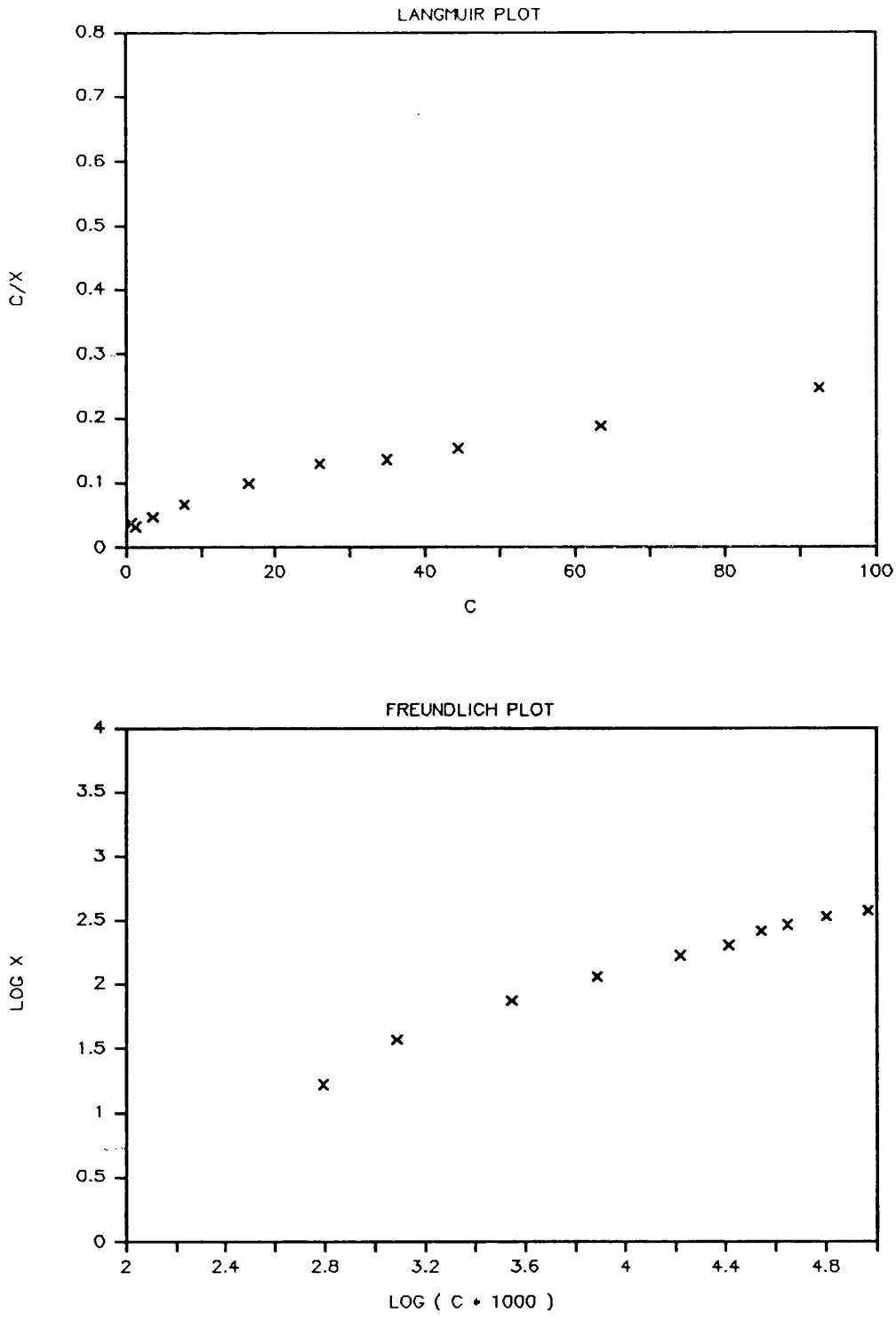


Fig. 4.13 Langmuir and Freundlich isotherms for the Dreghorn No.2 A soil.

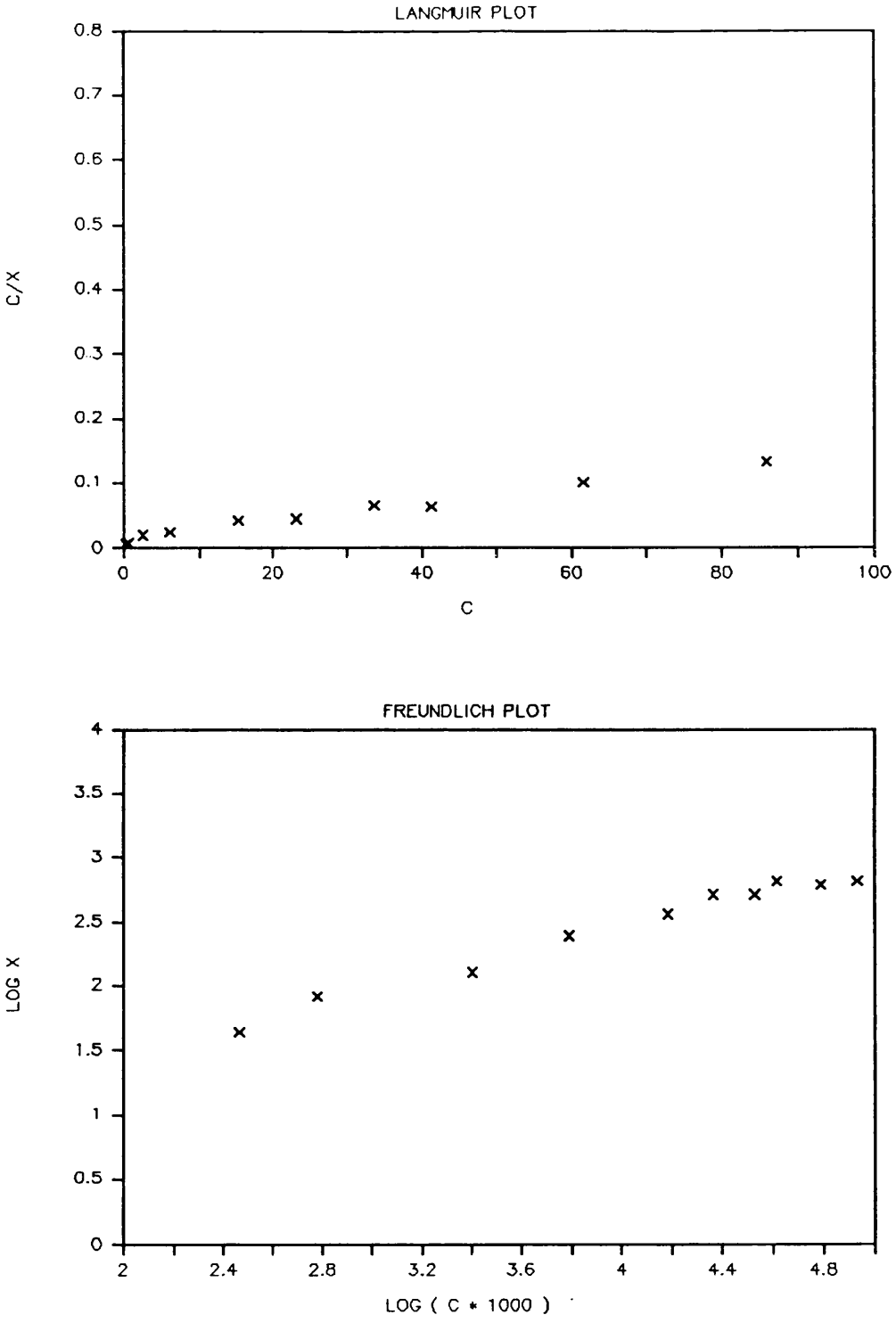


Fig. 4.14 Langmuir and Freundlich isotherms for the Dreghorn No.2 B soil.

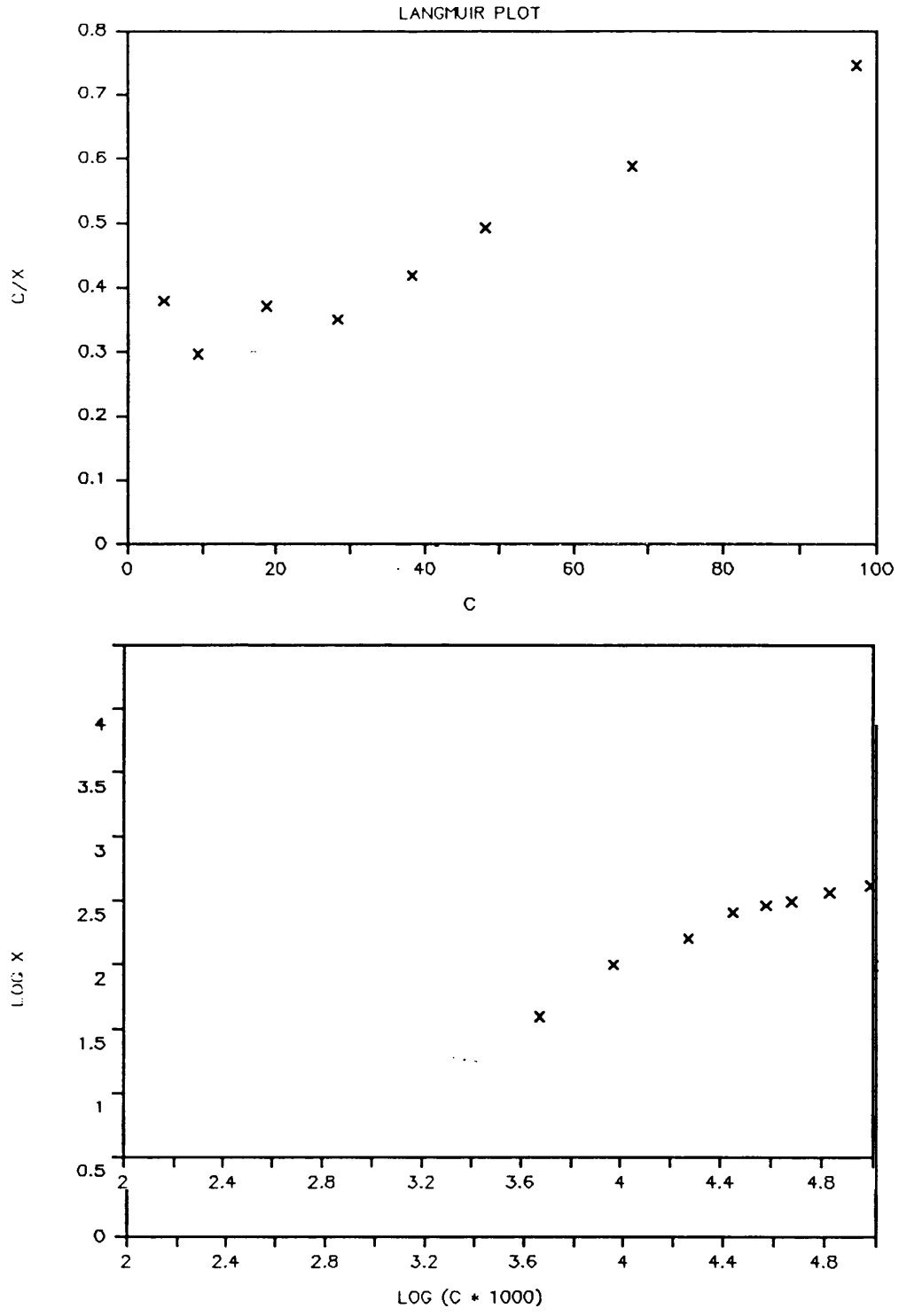


Fig. 4.15 Langmuir and Freundlich isotherms for the Midelney (Grass) A soil.

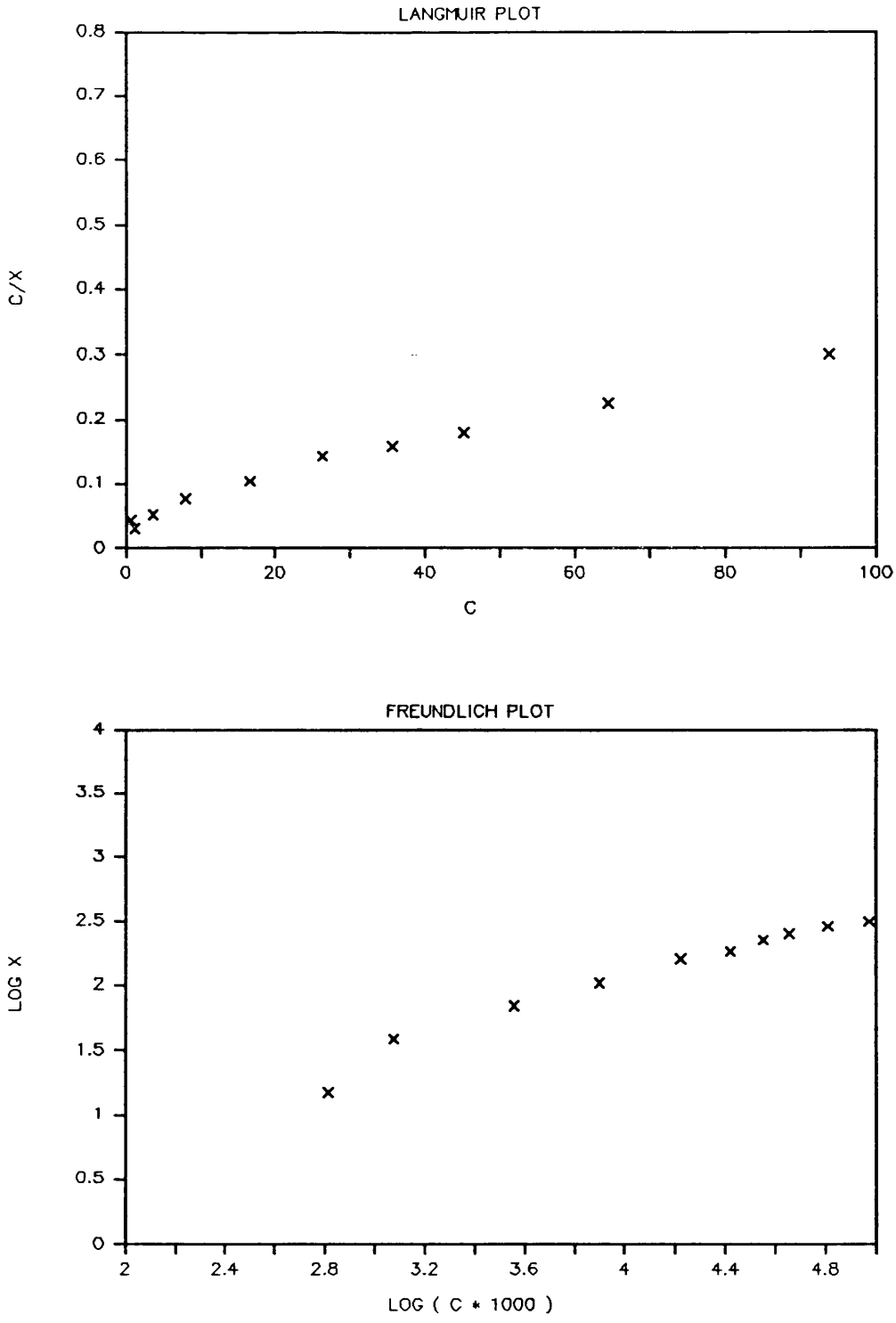


Fig. 4.16 Langmuir and Freundlich isotherms for the Midelney (Arable) A soil.

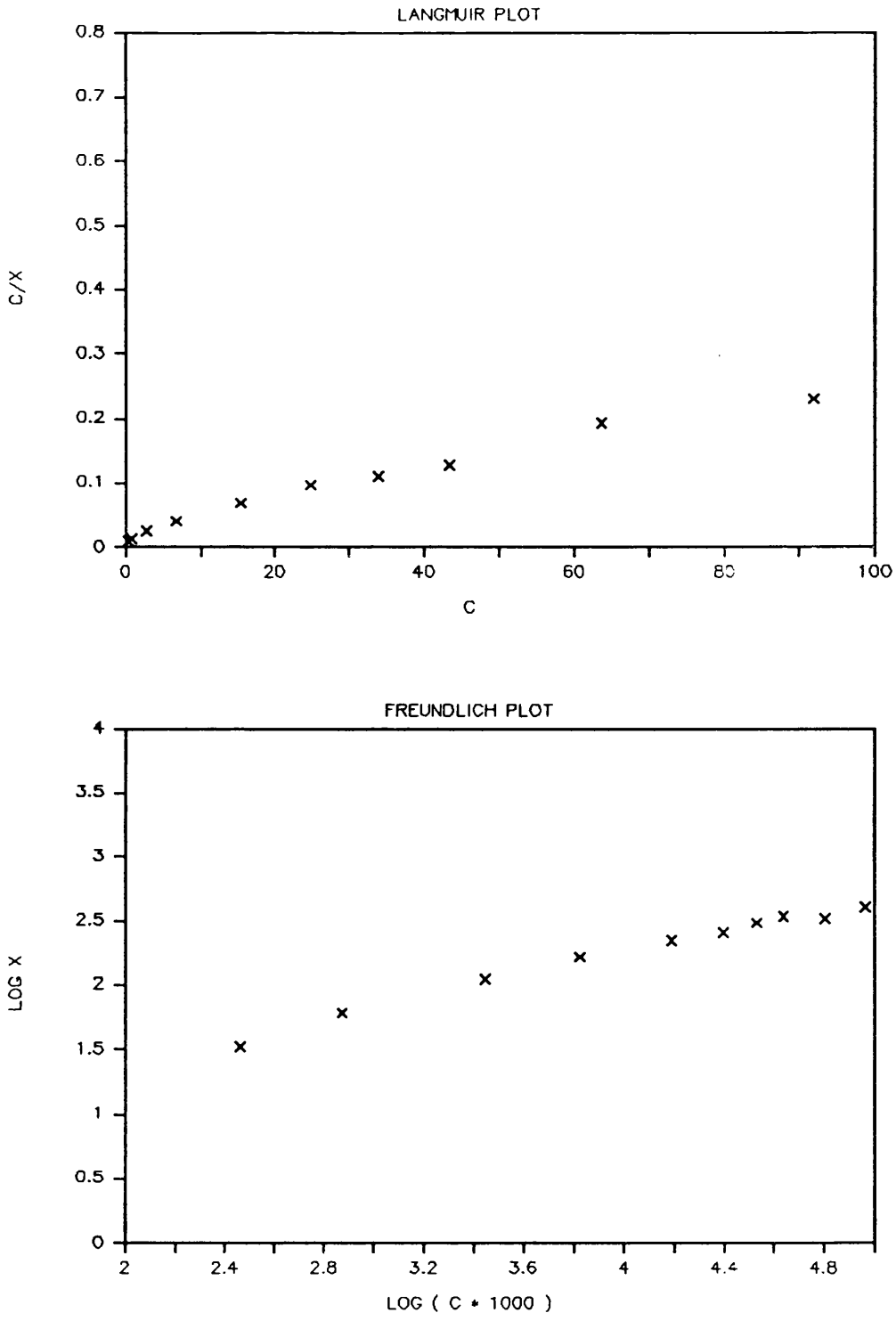


Fig. 4.17 Langmuir and Freundlich isotherms for the Midelney (Arable) B soil.

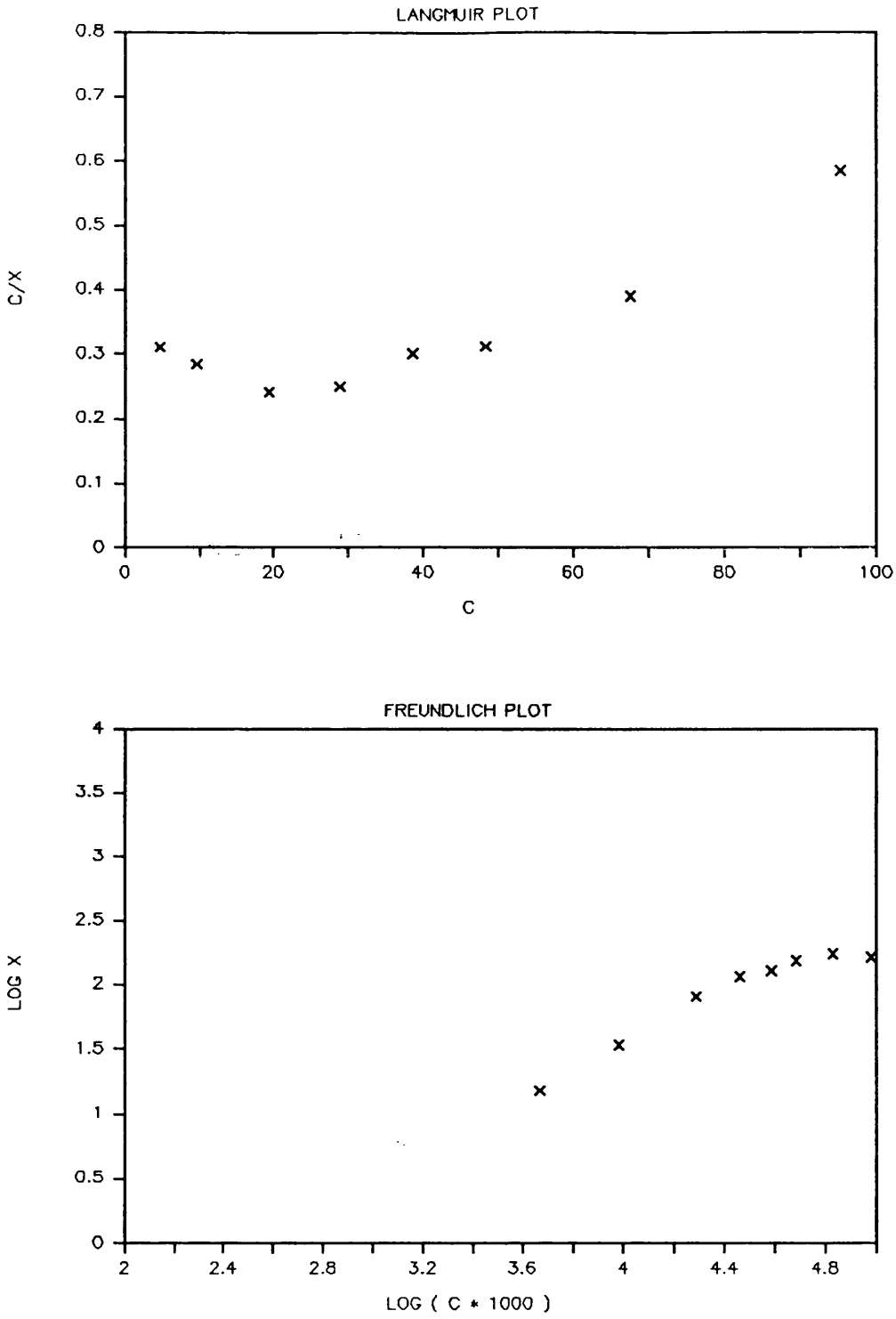


Fig. 4.18 Langmuir and Freundlich isotherms for the Alluvium soil.

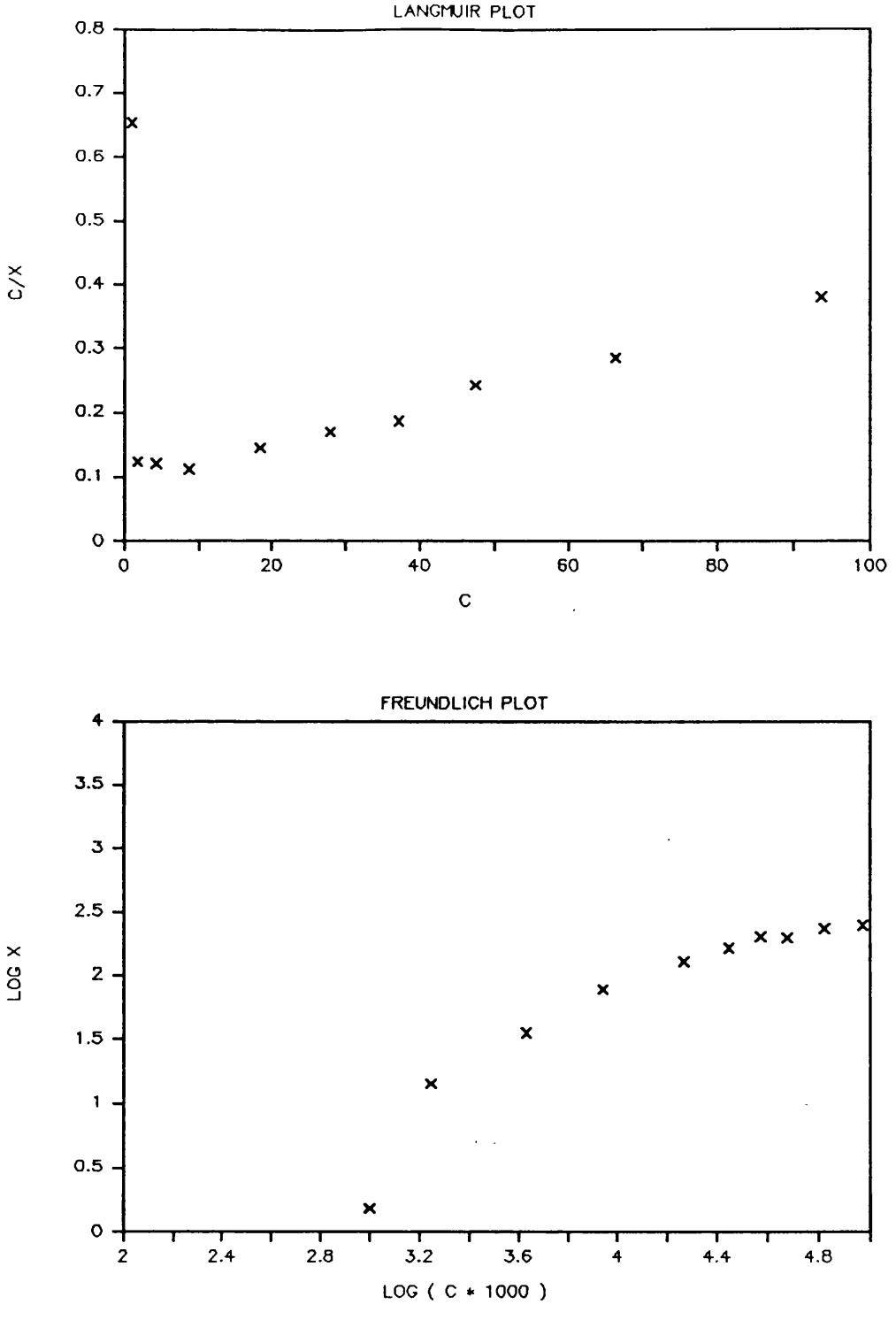


Fig. 4.19 Langmuir and Freundlich isotherms for the Swaffham Prior A soil.

When the Langmuir equation was applied to the soil P adsorption data, by plotting the values of transformed adsorbed P ,C/X, against C, the equilibrium concentration of P, the soils behaved differently. Some of the soils conformed well to the Langmuir equation, while others showed a slight curving at the lower end as in Dreghorn No.1 A soil. The bending of the lines down toward the x-axis was thought to be due to the strong adsorbing sites, which adsorbed most of the P at lower P equilibrium concentration (0-2 μg P cm^{-3}). On the other hand, the upward bending showed relatively less P adsorption as in case of Midelney (Grass) soil. It might be due to the occupation of adsorbing surfaces by native or residual phosphate.

Using the R^2 values as a test for goodness of fit, only the Dunlop A, Peat (Blackstoun), Midelney (Arable) A and Swaffham Prior A soils appeared to conform relatively well to the Langmuir equation (Table 4.2). The rest of the soils did not properly follow the Langmuir equation, indicating that phosphate adsorption did not occur by a single process in these soils.

The reason for the data not conforming to the Langmuir according to Bache and Williams (1971) is that adsorbed phosphate may have migrated to the sub-surface layers. They also pointed out that the Langmuir equation can be used to characterize the adsorption only over limited concentration ranges.

Table 4.2 Phosphorus adsorption constants for soils obtained from Langmuir and Freundlich plots:

Soils	Langmuir plots			Freundlich plots		
	adsorption maximum $\mu g\ g^{-1}$	bonding energy $cm^3 \mu g^{-1}$	R^2 %	Slope	intercept	R^2 %
Dreghorn No.1 A	553	0.053	95.1	0.557	43.86	98.6
Dreghorn No.1 B	518	0.095	96.6	0.432	76.14	99.1
Darvel A	900	0.061	96.9	0.578	71.05	99.3
Capring-ton A	474	0.028	97.4	0.736	15.76	97.3
Bargour A	566	0.022	93.4	0.755	15.91	95.4
Dunlop A	1532	0.097	98.8	0.578	148.73	97.5
Peat(Bla-ckstoun)A	849	0.087	98.8	0.596	73.89	96.7
Dreghorn No.2 A	440	0.048	96.7	0.598	29.42	98.1
Dreghorn No.2 B	708	0.111	98.4	0.485	92.62	97.8
Midelney (Grass) A	221	0.016	92.2	0.763	5.00	94.9
Midelney (arable) A	357	0.057	98.0	0.570	28.35	96.7
Midelney (Arable) B	406	0.112	97.6	0.422	65.72	98.5
Alluvium A	321	0.015	73.5	0.838	5.32	93.0
Swaffham Prior A	1012	0.005	3.3	0.978	5.15	87.8

Some workers suggested that adsorption follows the Langmuir isotherm in the range of 25-100 μg g^{-1} of adsorbed P (Cole et al., 1953; Kuo and Lotse, 1972; Holford and Mattingly, 1975). Harter and Baker (1977) considered the effect of desorbed ions in the equilibrium solution as the cause of the commonly reported curvilinear nature of the simple Langmuir isotherm. They further stated that when a second species is desorbed, reactions of the adsorbate with the surface will be altered, depending upon the concentration of the desorbed species in the solution. Poor fits of the simple Langmuir isotherm to adsorption data have been attributed to the presence of different types of sites on the adsorbent surface (Syers et al., 1973; Posner and Bowden, 1980).

The Freundlich equation was applied to the same P adsorption data, by plotting logarithmic values of X, the adsorbed P, against logarithmic values of C, the equilibrium P concentration. Using the R^2 value as a test for goodness of fit, the Freundlich equation describes adequately phosphate retention by most of the soils studied (Table 4.2). In some of the soils the line became gently curved at higher P concentrations which indicates no further P adsorption at this point, as in Dunlop A, Peat (Blackstoun), Dreghorn No.2 B and Darvel A soils. The Freundlich equation showed another characteristic in that the adsorption points were compressed at the top end, which might have disguised the poor linearity. Bache

and Williams (1971) and Barrow (1978) have reported gentle curves when the Freundlich equation was applied to soil phosphate adsorption data.

The Langmuir adsorption maximum and bonding energy constant were calculated for each soil. By comparing the Langmuir maximum values in Table 4.2 with the observed maximum values on P adsorption isotherms, it was revealed that in most of the soils the Langmuir maximum overestimated the P adsorbed on the plateau of Fig 4.1 to 4.5 (Table 4.1) except in Dreghorn No.1 B, Midelney(Arable) A and Midelney(Arable) B. The Langmuir adsorption maximum, closely related to a characteristic property of the soil i.e., its surface area (Olsen and Watanabe, 1957), has been reported to satisfactorily characterize the P adsorption properties of soils (Rajan, 1973).

The binding constants, derived from the slope and intercept of Langmuir equation, for P adsorption by the soils, ranged from 0.005 to 0.112 cm^3 μg^{-1}. It is evident from the data that the values of binding constant are relatively higher in sub-surface soils than the corresponding surface soils, indicating more affinity for P in the sub-surface soils. As the value of the constant increases, the bonding energy of the soil for phosphorus increases. Thus the cumulative effects of organic matter, by blocking adsorption sites (Le Mare, 1981) and residual effect of P-fertilizer (Fox and Xue-yuan, 1986) may have

reduced the affinity for P in the surface soils. As the binding constant is markedly affected by experimental error (Rennie and McKercher, 1959), little weight could be placed on the differences obtained. They obtained an average value of 0.141 cm^3 μg^{-1} for this constant, while Olsen and Watanabe (1957) reported 0.92 and 4.39 cm^3 μg^{-1} for alkaline and acidic soils respectively. A similar range of the binding constant, 0.91-4.31 cm^3 μg^{-1} has been reported by Oskay (1986).

The Langmuir adsorption maximum has been reported as the most important parameter for characterizing the P adsorption properties of soils (Rajan, 1973). Due to this parameter, the use of Langmuir is preferred to other conventional equations. There was a wide range in the P adsorption maxima (Table 4.2), which was to be expected in view of the differences in soil properties. The Dunlop A soil had the largest adsorption maximum of 1532 $\mu g\ g^{-1}$. This soil had the lowest pH of 4.9 of all soils used, which could favour the P adsorption. It also contained the highest clay and oxalate extractable Fe content in the Scottish soils, which could be the cause of more adsorption. Kato and Owa (1989) have also reported higher P adsorption capacity in clayey soil than in sandy soil. The second highest P adsorption maximum of 900 $\mu g\ g^{-1}$ was recorded in Darvel A soil. This soil was sandy clay loam in texture. In the third position was Peat(Blackstoun) A which had the P adsorption maximum equal to 849 $\mu g\ g^{-1}$.

The Peat(Blackstoun) A had the highest organic matter content of the soils studied. The lowest adsorption maximum of P was obtained in Midelney (Grass) A. Which means the adsorption sites in this soil did not retain more phosphate and as such it is more available to plants.

To improve the R^2 values for goodness of fit, some of the P adsorption points at the lower end for the Langmuir and at the top for the Freundlich equation were excluded from the calculations. On the amended P adsorption data both equations were applied and the constants were calculated (Table 4.3). It was observed that in most soils the amended data conformed relatively well to both equations (Fig 4.20 to 4.33) and R^2 values were improved. By this practice the limitations of the Langmuir equation at the lower end and for Freundlich equation at the top end were also revealed.

Table 4.3 Phosphorus adsorption constants for soils obtained from Langmuir and Freundlich amended plots:

Soils	Langmuir plots			Freundlich plots		
	adsorption maximum $\mu g\ g^{-1}$	bonding energy $cm^3\ \mu g^{-1}$	R^2 %	Slope	intercept	R^2 %
Dreghorn No.1 A	603	0.039	95.7	0.557	43.86	98.6
Dreghorn No.1 B	560	0.062	97.2	0.441	75.60	99.3
Darvel A	973	0.045	98.1	0.596	69.95	99.8
Capring-ton A	474	0.028	97.4	0.864	13.69	98.8
Bargour A	539	0.025	94.2	0.798	14.64	96.2
Dunlop A	1606	0.076	99.5	0.627	146.25	98.9
Peat(Bla-ckstoun)A	849	0.087	98.8	0.678	69.56	99.0
Dreghorn No.2 A	473	0.037	97.4	0.616	28.82	98.3
Dreghorn No.2 B	748	0.080	98.9	0.520	90.82	98.8
Midelney (Grass) A	196	0.021	98.1	0.826	4.24	96.2
Midelney (arable) A	395	0.039	99.2	0.590	27.67	96.9
Midelney (Arable) B	431	0.078	98.0	0.448	64.68	99.2
Alluvium A	221	0.037	94.9	1.013	3.41	98.5
Swaffham Prior A	319	0.038	99.3	1.109	4.29	89.3

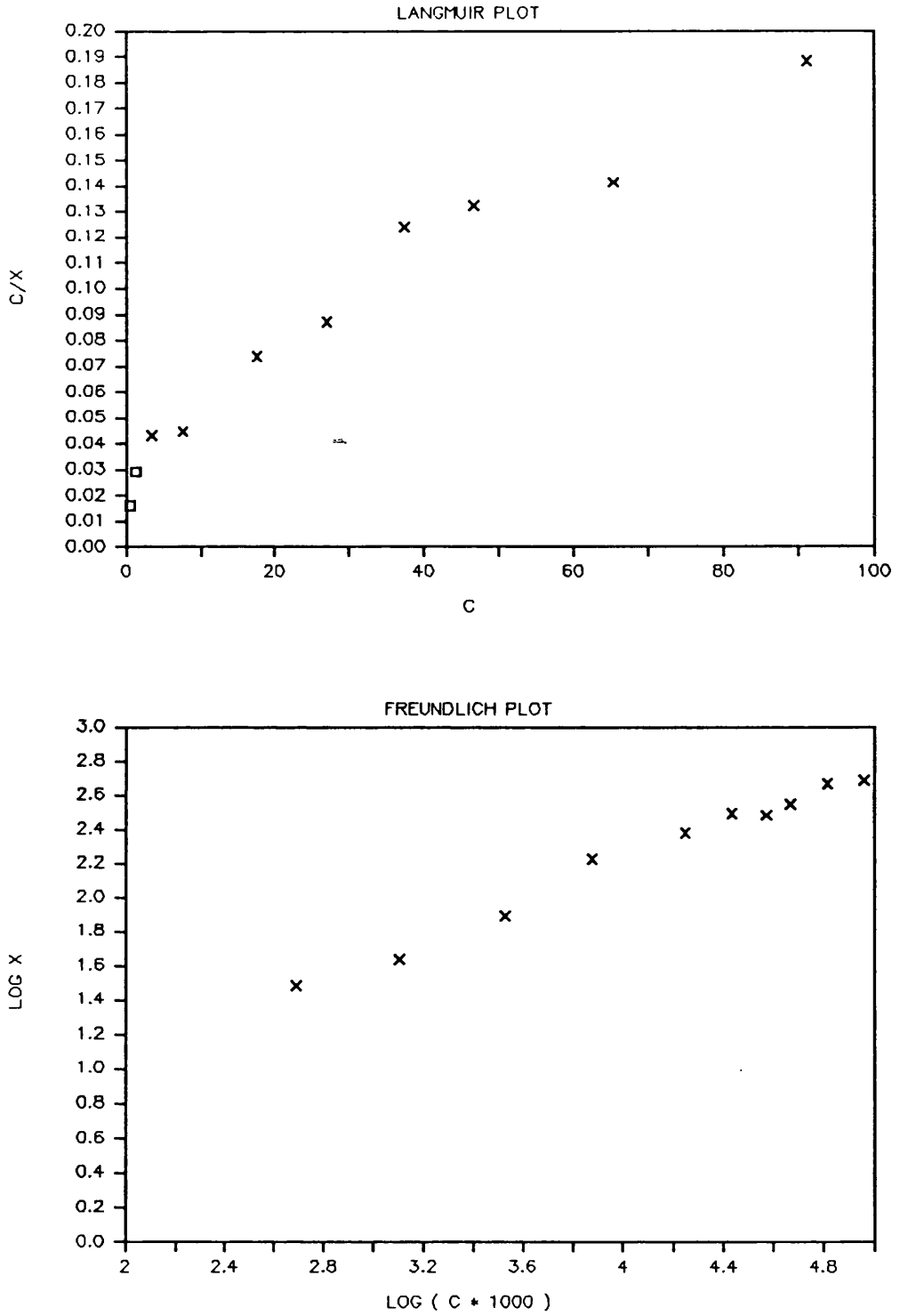


Fig. 4.20 Langmuir and Freundlich isotherms for the Dreghorn No.1 A soil.

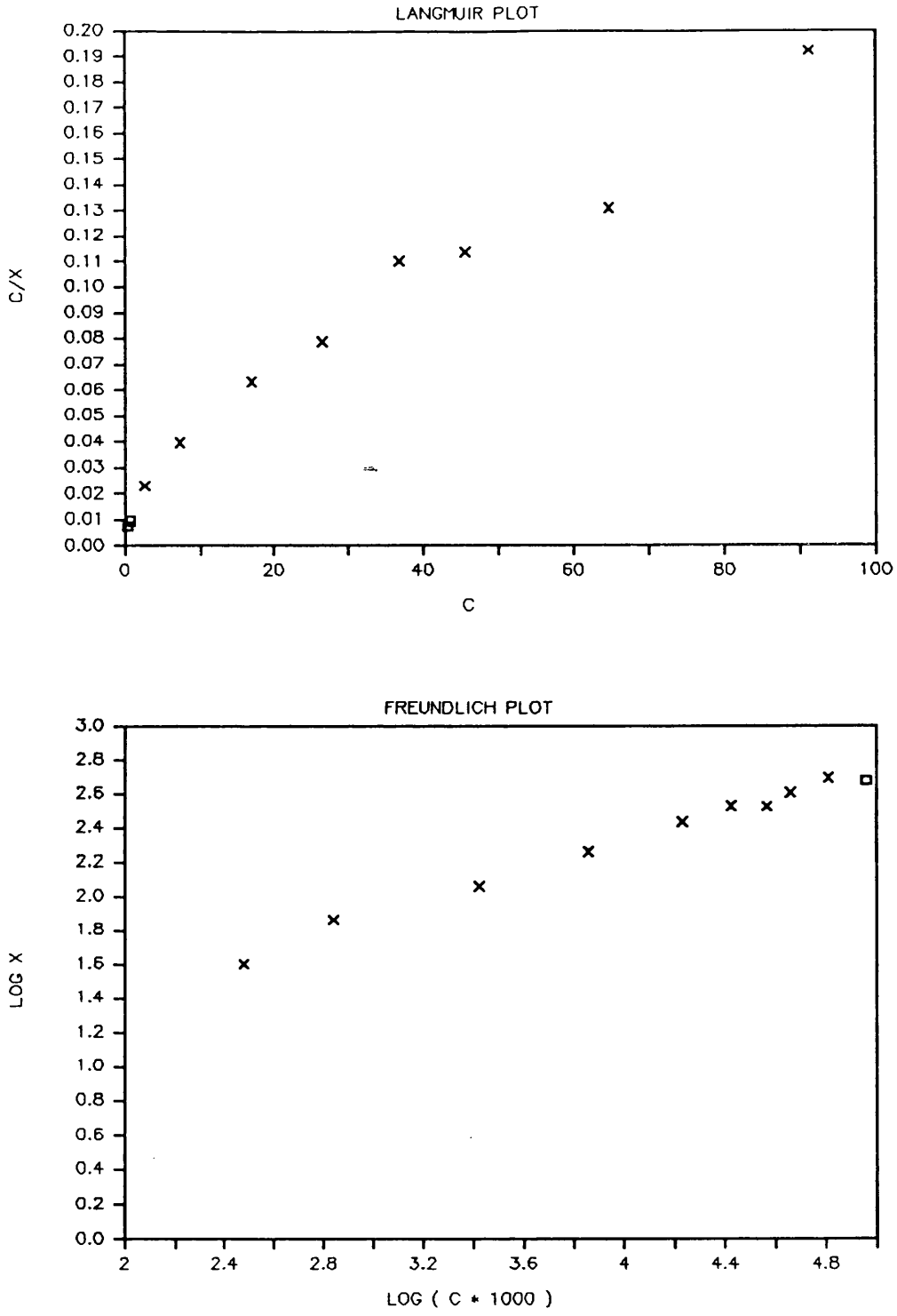


Fig. 4.21 Langmuir and Freundlich isotherms for the Dreghorn No.1 B soil.

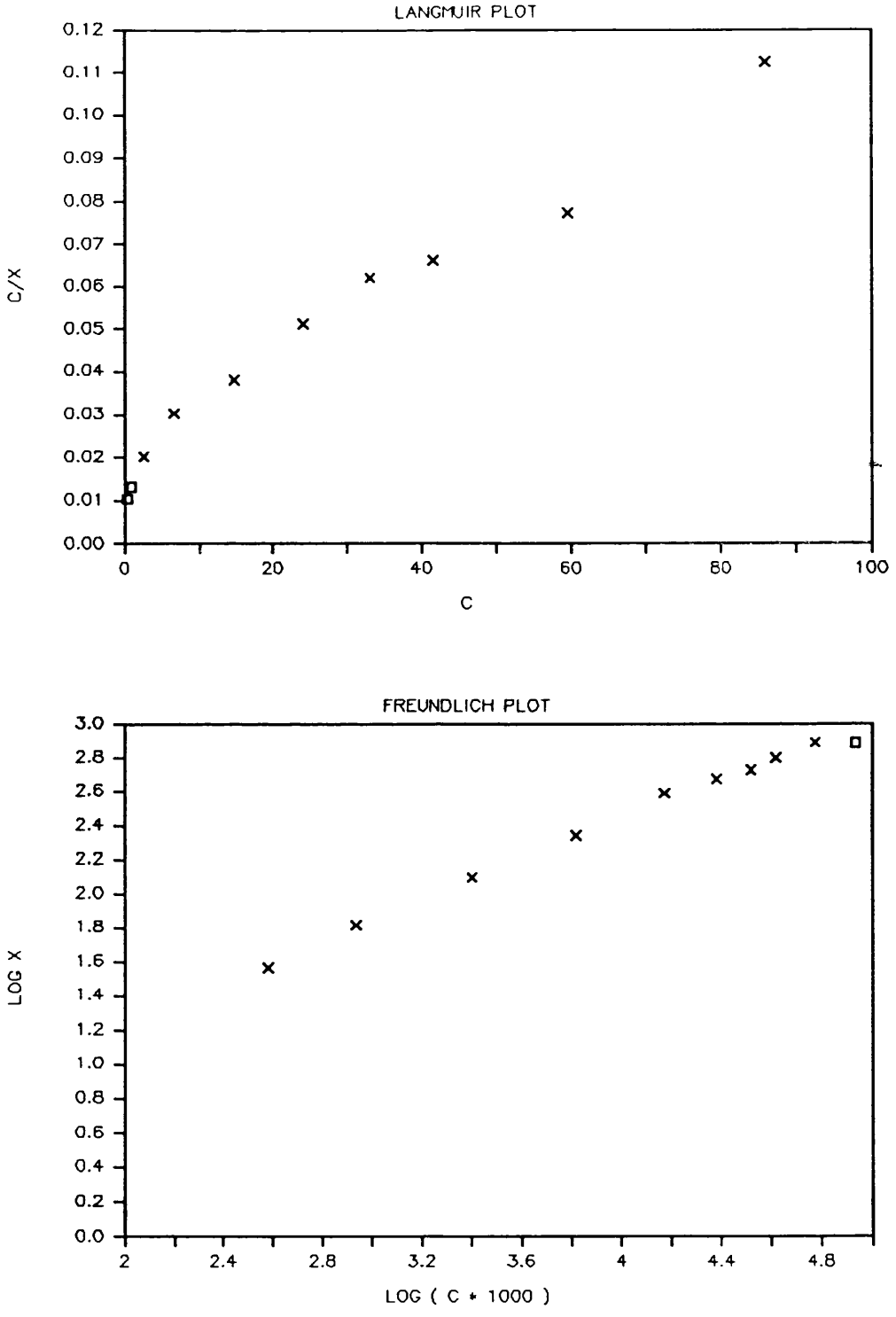


Fig. 4.22 Langmuir and Freundlich isotherms for the Darvel A soil.

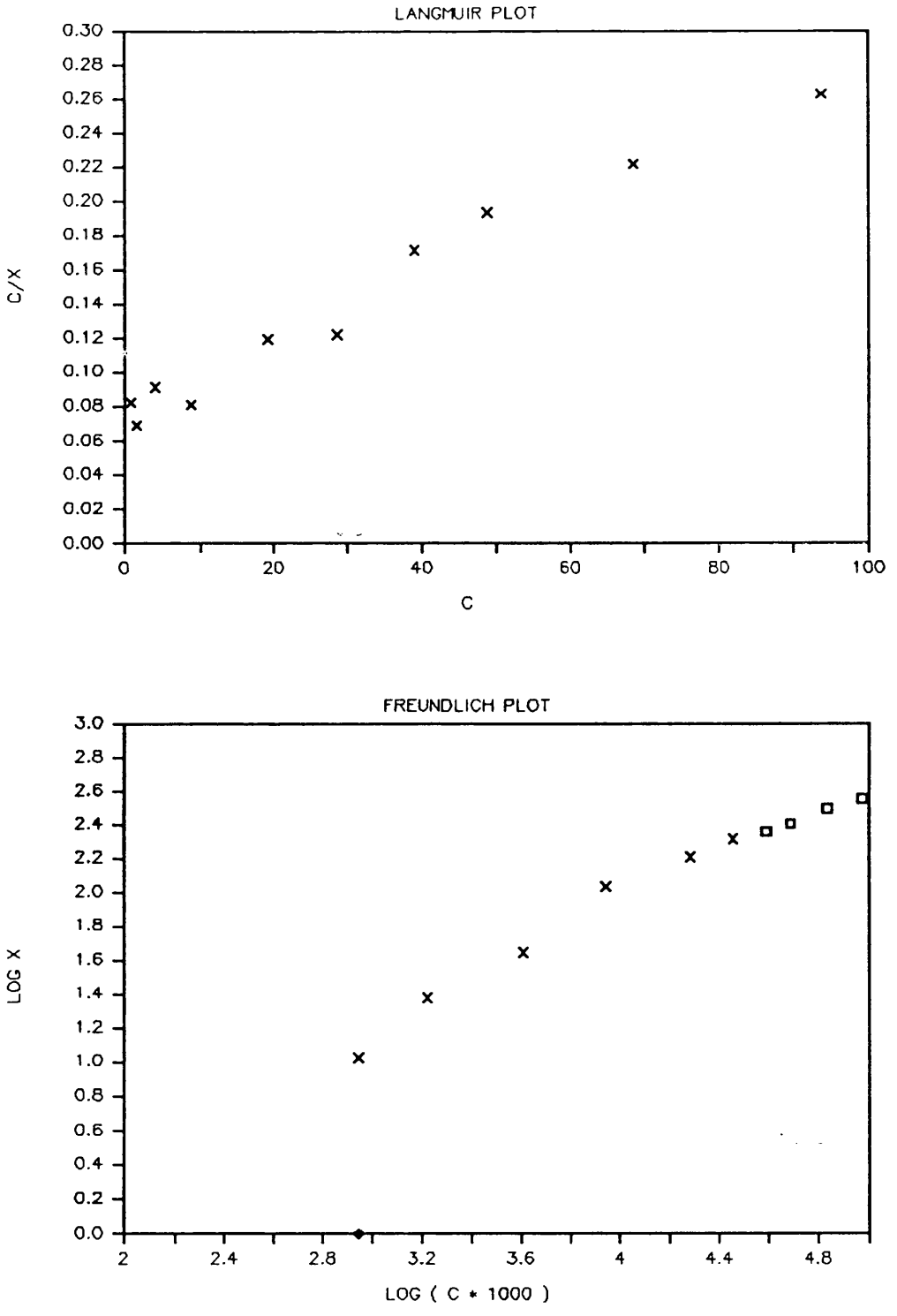


Fig. 4.23 Langmuir and Freundlich isotherms for the Caprington A soil.

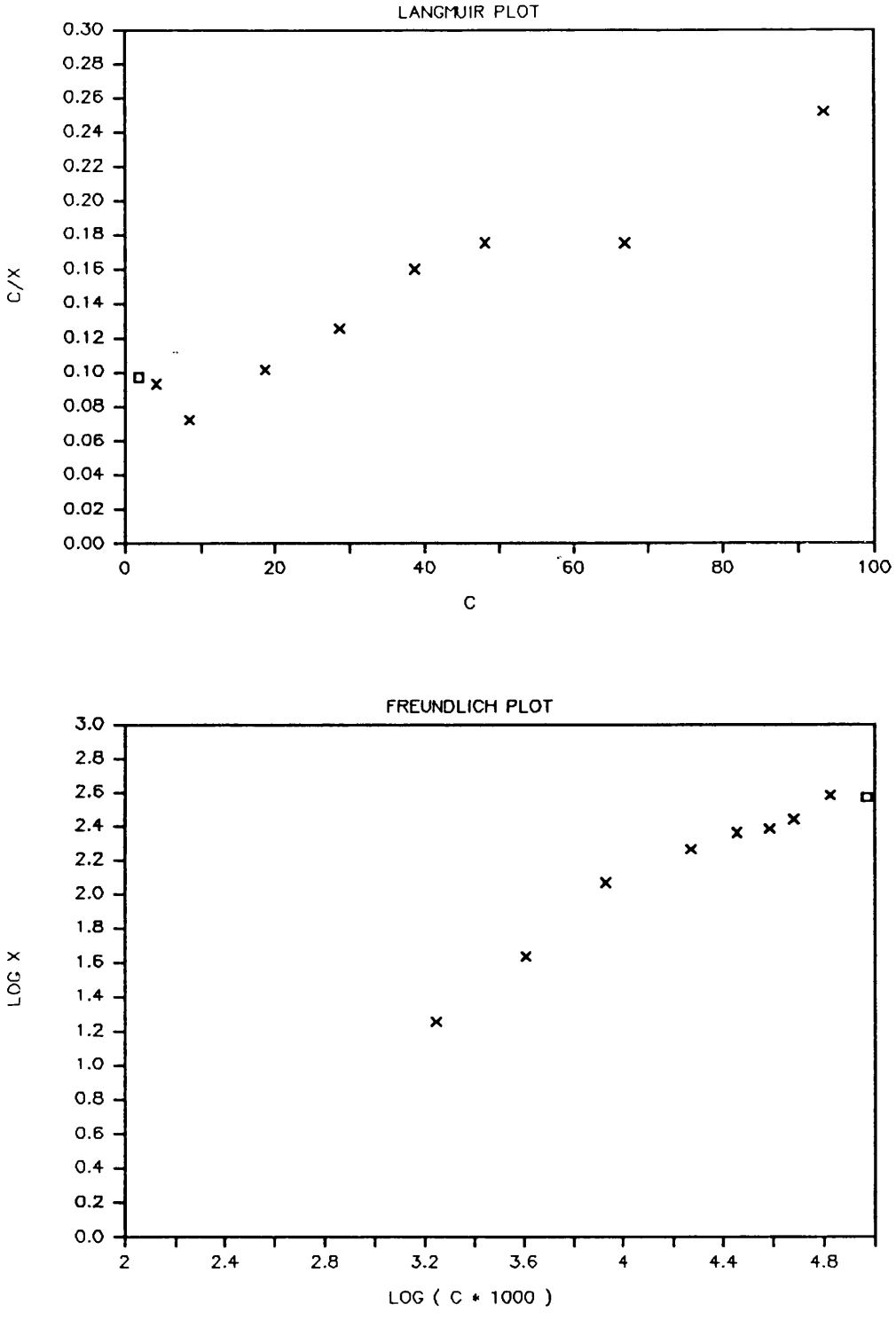


Fig. 4.24 Langmuir and Freundlich isotherms for the Bargour A soil.

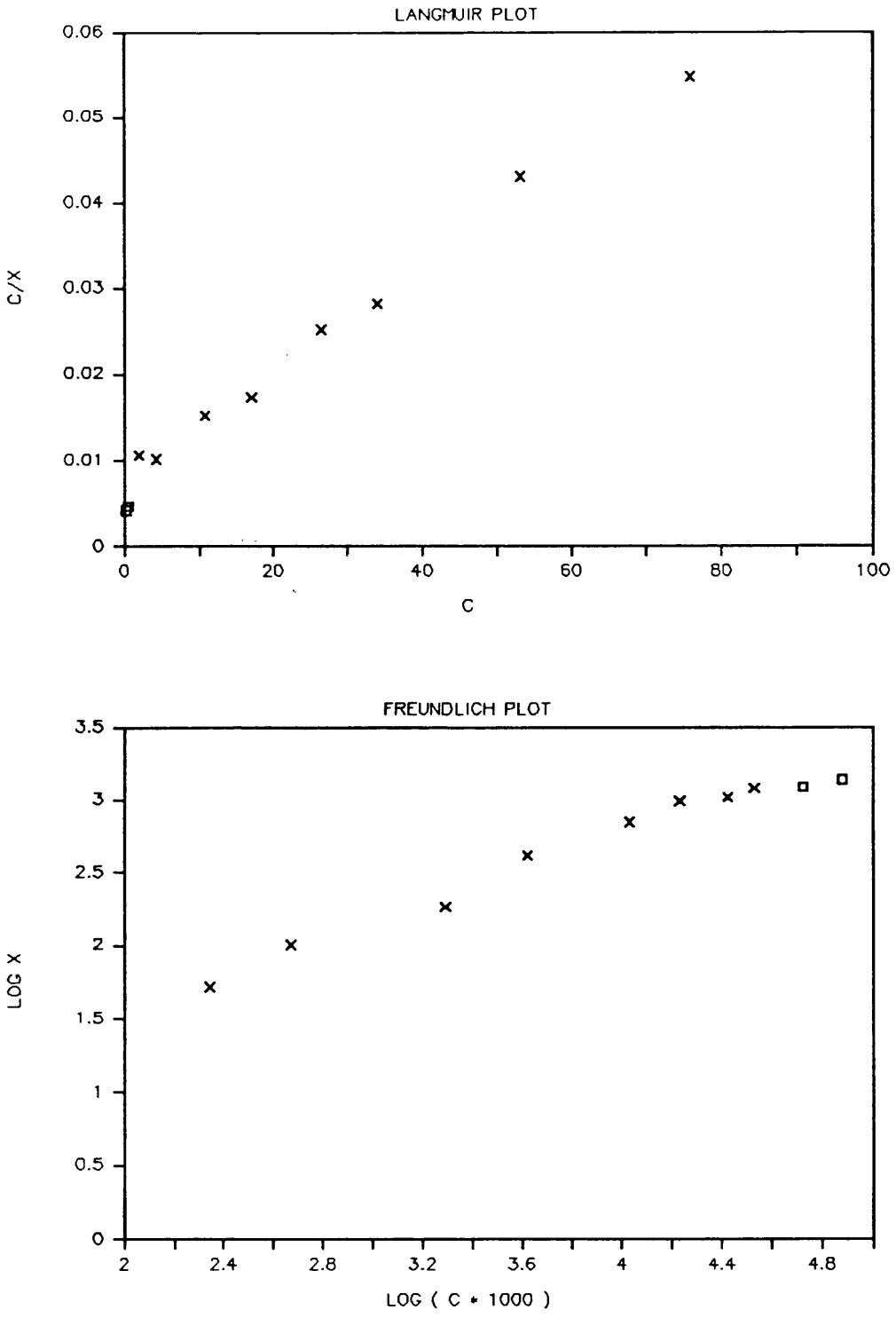


Fig. 4.25 Langmuir and Freundlich isotherms for the Dunlop A soil.

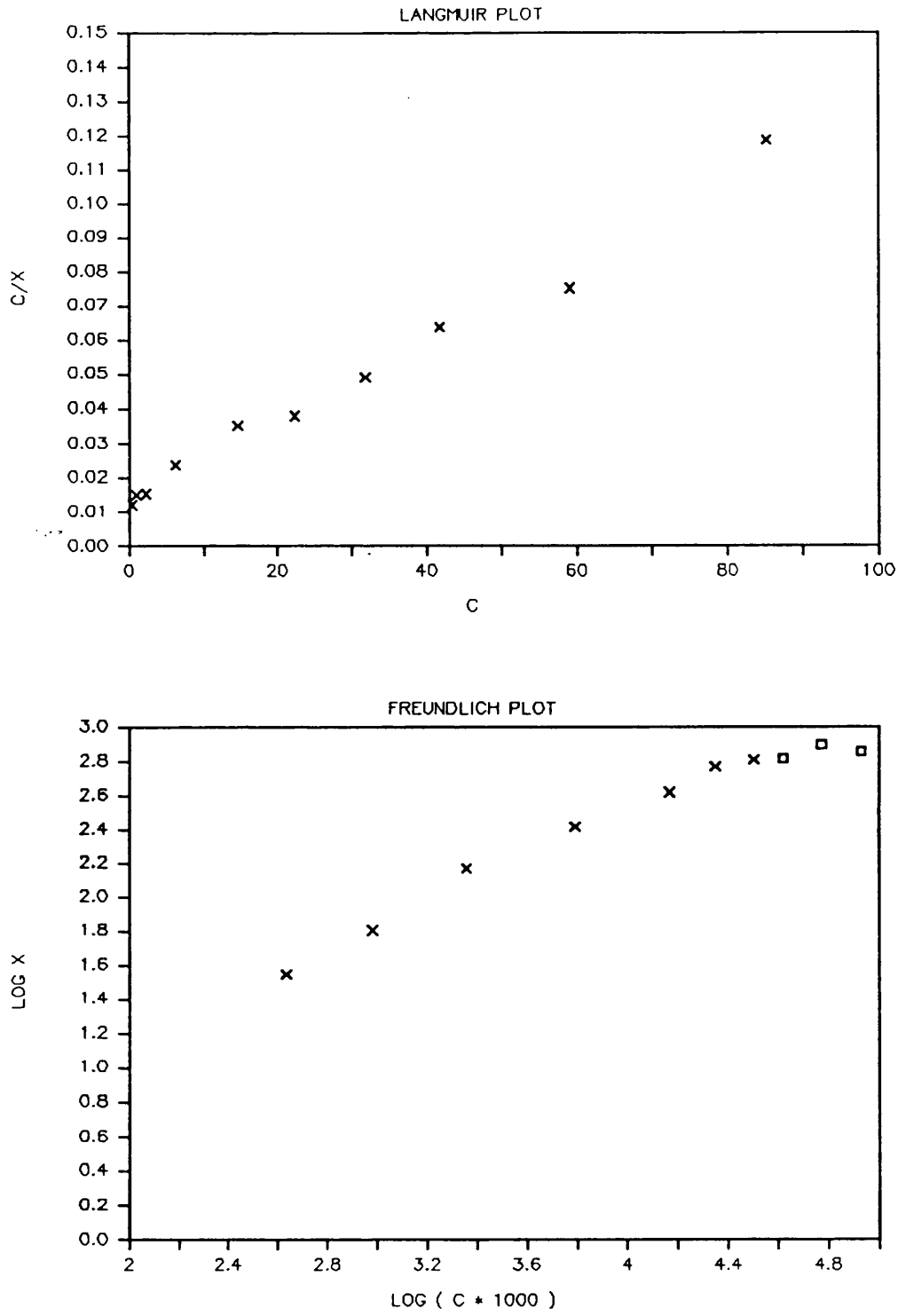


Fig. 4.26 Langmuir and Freundlich isotherms for the Peat (Blackstoun) soil.

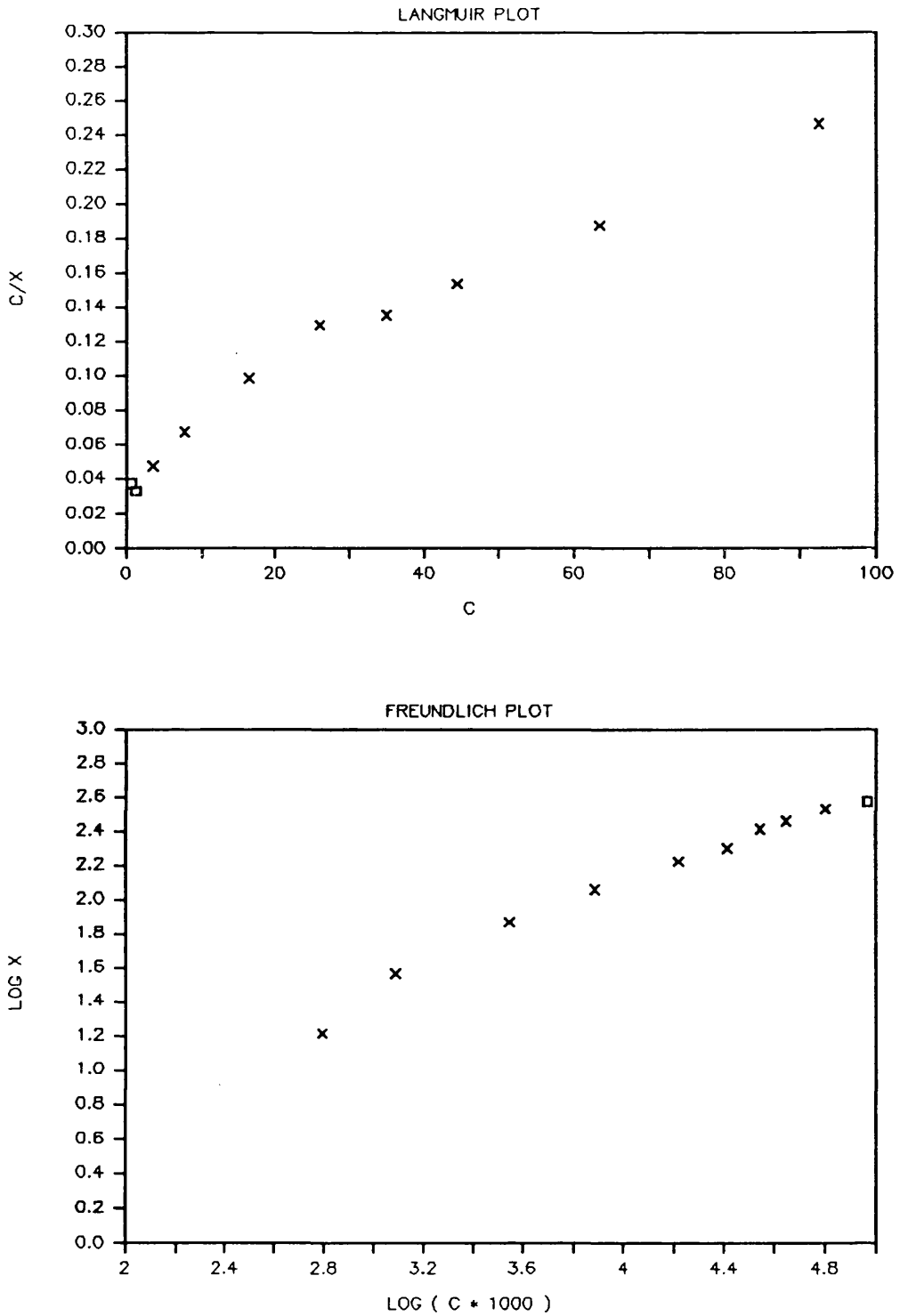


Fig. 4.27 Langmuir and Freundlich isotherms for the Dreghorn No.2 A soil.

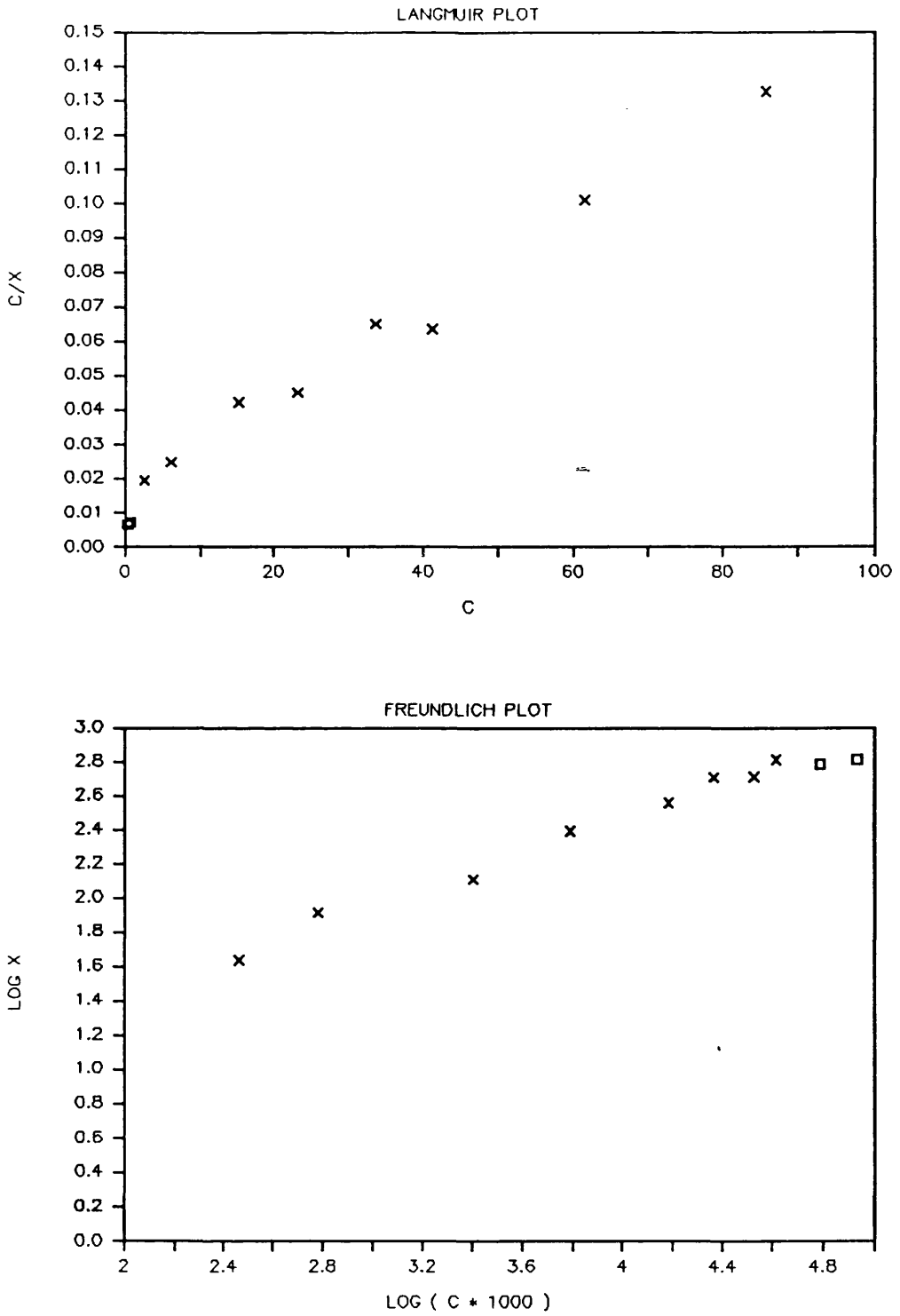


Fig. 4.28 Langmuir and Freundlich isotherms for the Dreghorn No.2 B soil.

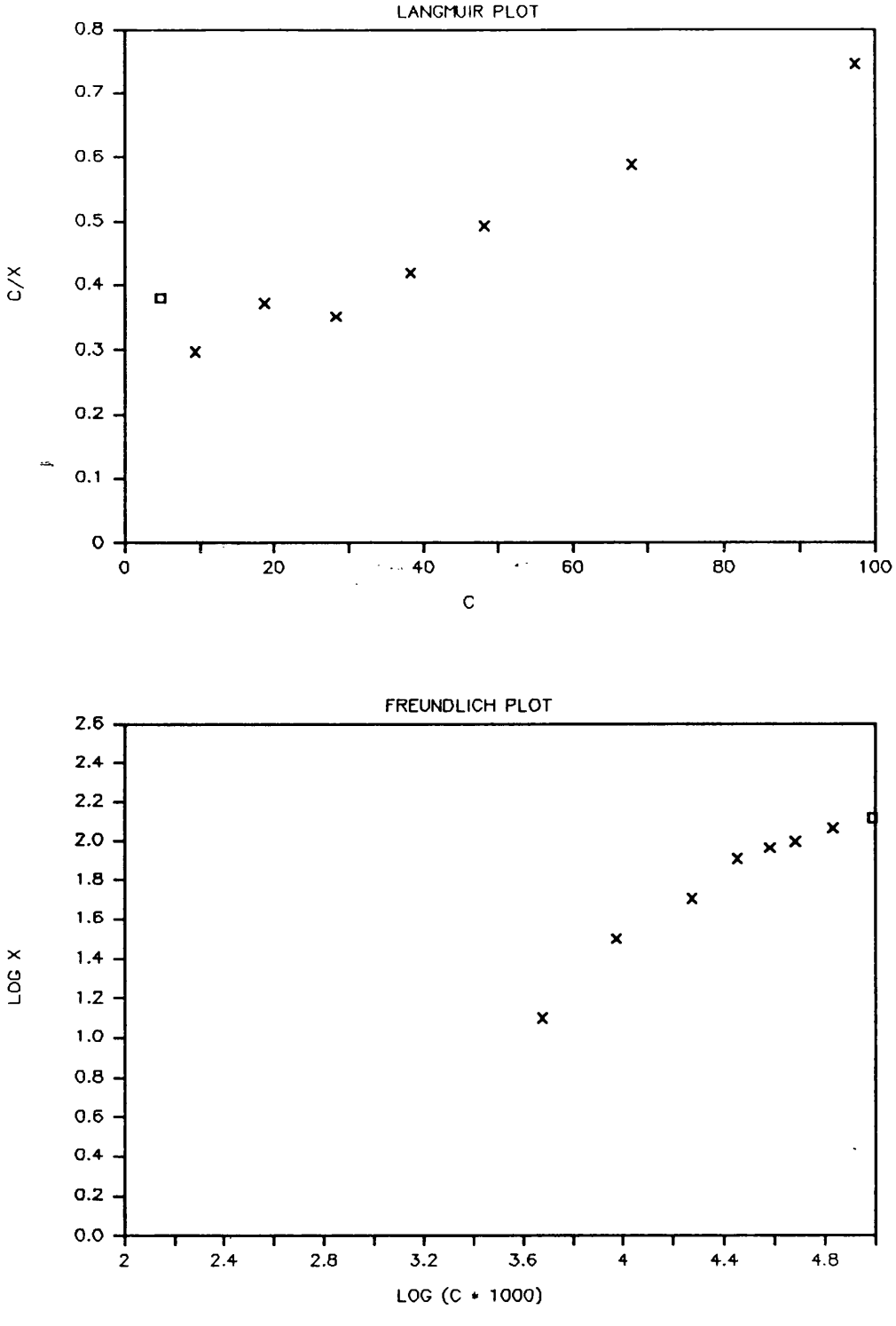


Fig. 4.29 Langmuir and Freundlich isotherms for the Midelney (Grass) A soil.

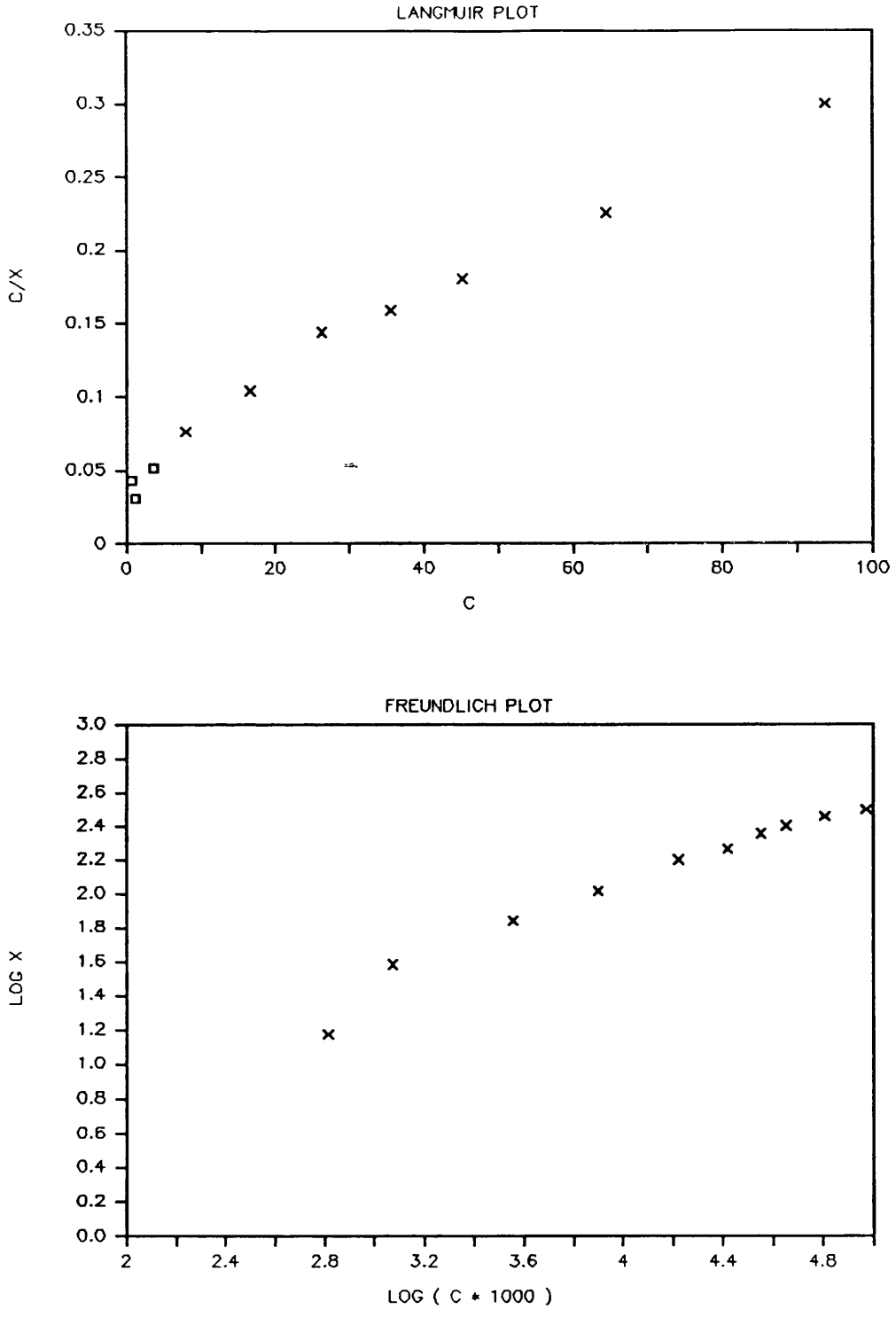


Fig. 4.30 Langmuir and Freundlich isotherms for the Midelney (Arable) A soil.

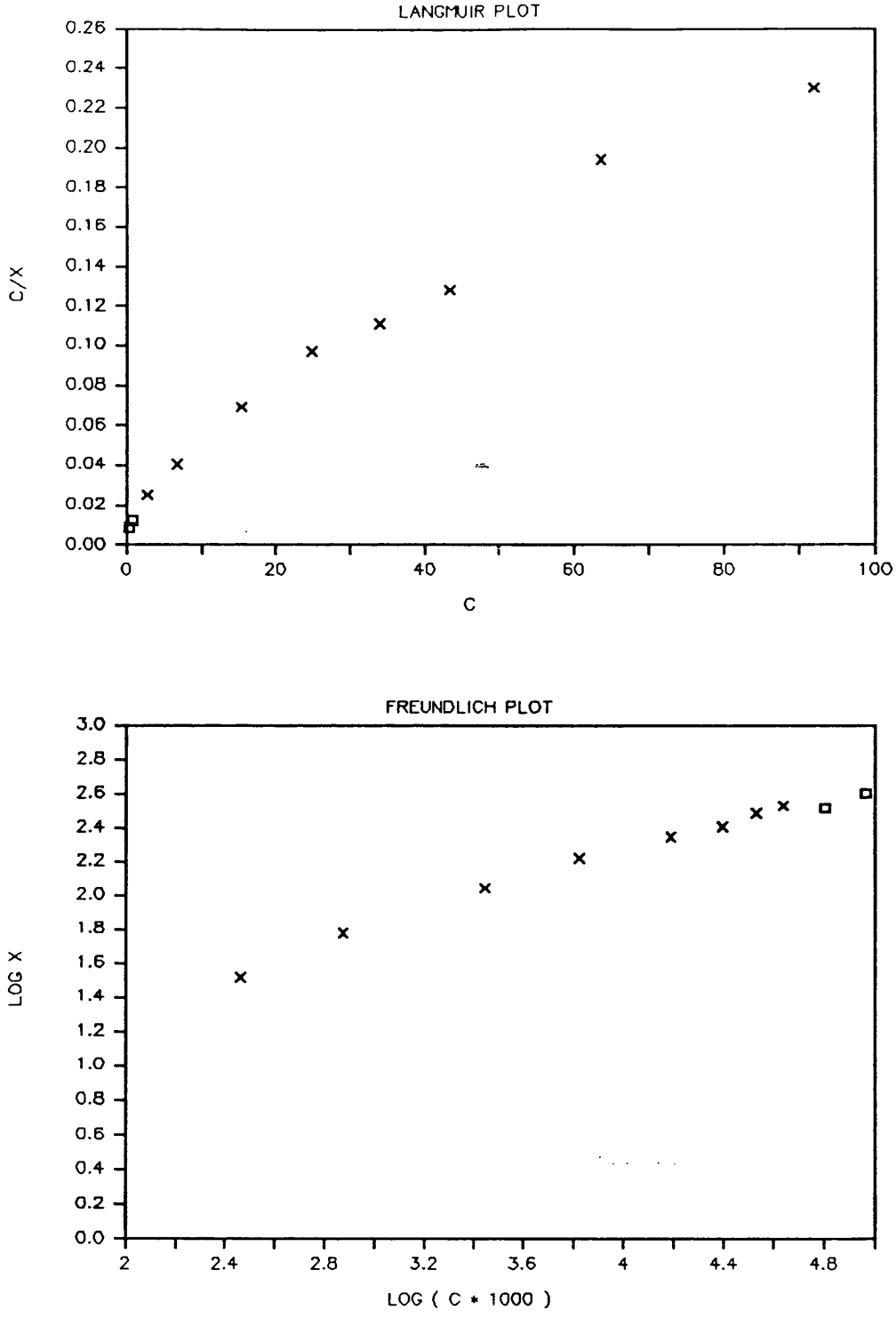


Fig. 4.31 Langmuir and Freundlich isotherms for the Midelney (Arable) B soil.

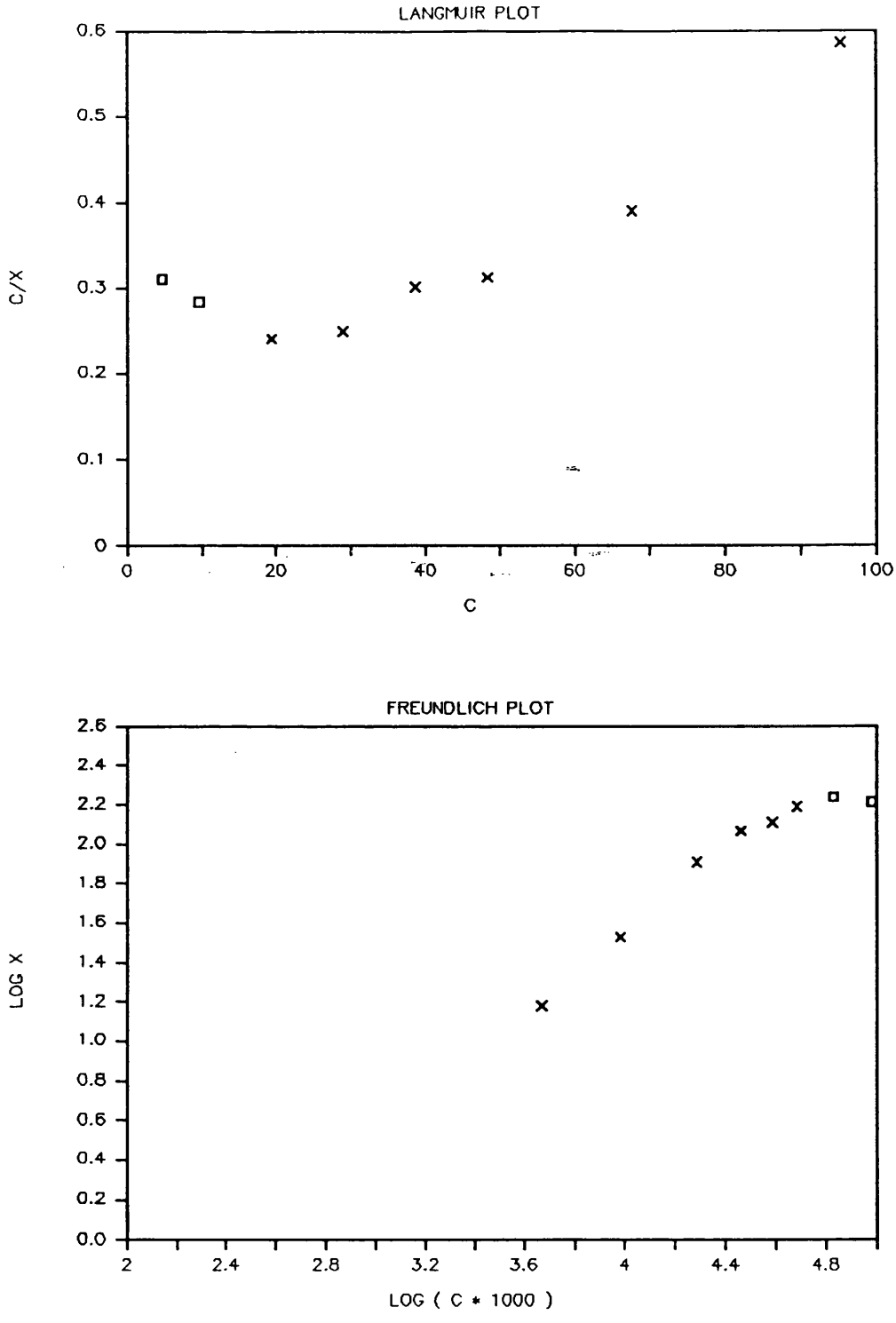


Fig. 4.32 Langmuir and Freundlich isotherms for the Alluvium A soil.

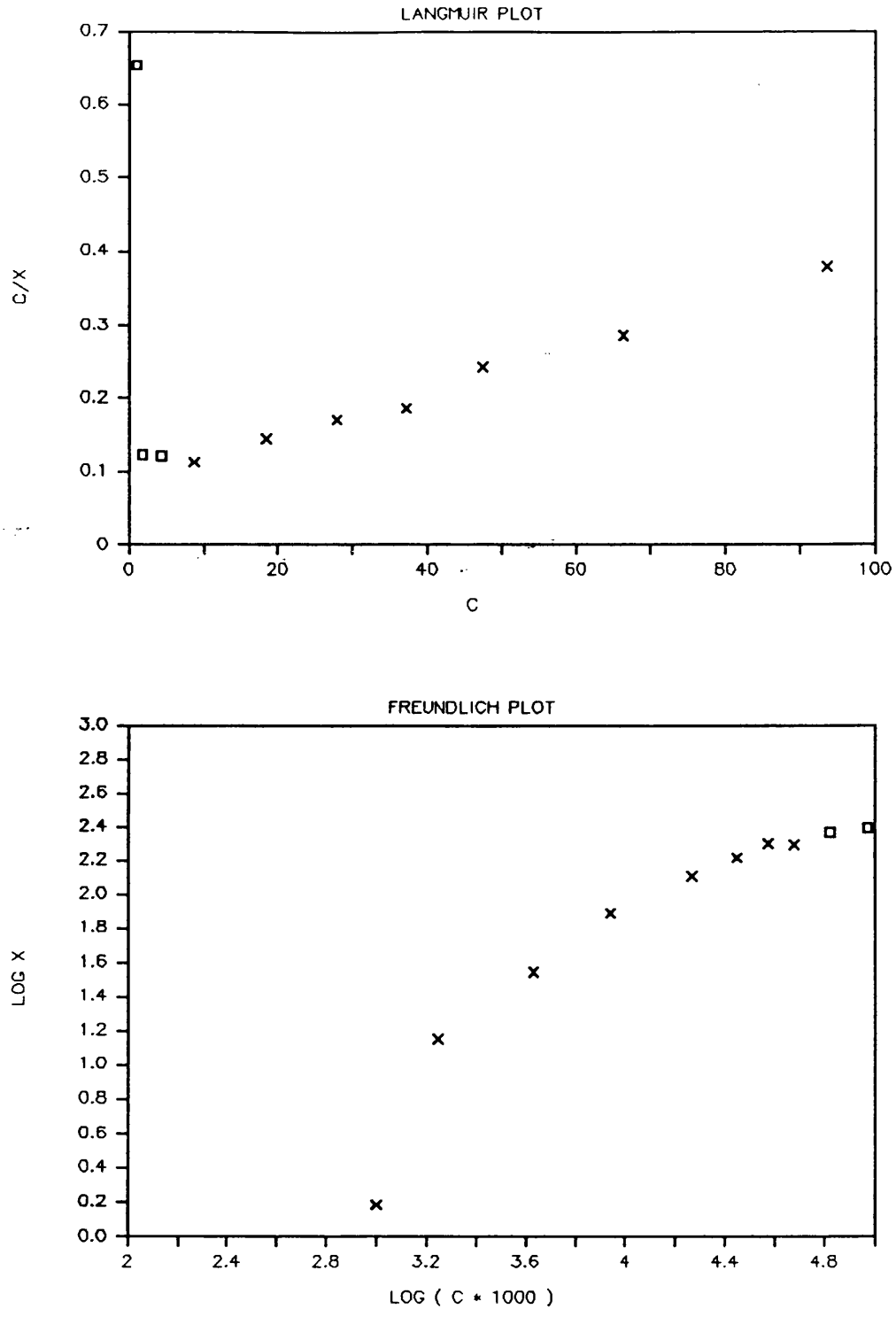


Fig. 4.33 Langmuir and Freundlich isotherms for the Swaffham Prior A soil.

4.3.3 Relationship between Langmuir maximum and some soil properties:

The relationships between Langmuir adsorption maximum and some soil properties were studied. Coefficients of correlation for all soil samples, Scottish soils and English soils are given in Table 4.4.

Table 4.4 Correlation Coefficients (r) Between Langmuir Maximum and Some Soil Properties:

Soil properties	All soils n = 14	Scottish soils n = 9	English soils n = 5
pH	-0.626*	-0.587ns	0.830ns
LOI %	0.487ns	0.694*	-0.706ns
Clay %	-0.080ns	0.764*	0.165ns
Fine Sand %	-0.161ns	-0.709*	-0.322ns
Oxalate extractable Al	0.613*	0.617ns	-0.631ns
Oxalate extractable Fe	0.822***	0.773*	0.042ns

* = P < 0.05 *** = P < 0.001 ns = P > 0.05

In all soils a very highly significant correlation was found between Langmuir maximum and oxalate extractable Fe. The same correlation was also significant in Scottish soils, while non-significant in English soils. Positive correlation of iron oxide with exchangeable P was reported by Le Mare (1981) in Nigerian soils. Shailaja and Sahrawat (1990) have given more importance to extractable iron for P adsorption than Al in the soils they studied. Oxalate extractable Al in all

soils was significantly correlated with Langmuir maximum ($r = 0.613^{*}$) and non-significantly correlated in split groups of soils. Pulford and Duncan (1975), Khalid et al., (1977), Cuttle (1983) and Adams et al., (1987) have reported close relationship between phosphate adsorption capacities of different soils and amounts of Al and/or Fe extracted by acid oxalate.

The pH of all soils was also significantly (negative) correlated with Langmuir maximum ($r = -0.626^{*}$) and non-significant correlation observed in the rest of the groups of soils. This suggests that the P adsorption was increased with decreasing pH. Olsen and Watanabe (1957) indicated that acid soils retained more phosphorus per unit of surface area and also held the phosphorus with greater energy than alkaline soils. Syers et al., (1973) also reported an increase of adsorbed P with decrease in soil pH. Imai et al., (1981) found that the P adsorption maximum was more at pH 5 than at pH 7 in allophanic soils. The clay, fine sand and LOI were significantly correlated with Langmuir adsorption maximum only in Scottish soils, while this relationships were found non-significant in the English soils and the whole group of soils. The significant relationships of clay and LOI with Langmuir adsorption maximum show that the P adsorption in these soils appears mainly associated with clay and organic matter. The results are consistent with the findings of Oskay (1986) for clay and Le Mare (1981)

for organic matter. Low and Black (1950) indicated that the initial reaction has been attributed to an exchange of phosphate ions for hydroxyl ions on the surfaces of soil particles. Though the clay content in some of the English soils was more than Scottish soils (Table 2.1), their P adsorption maximum was not significantly correlated with clay content. This indicates that there was no easy access of P to be adsorbed on clay surfaces in these soils. Fox and Kamprath (1970) suggested that the clay in iron oxide-cemented peds may become inactive. Poor correlation between P adsorption and clay content in the English soil, is consistent with the findings of Shailaja and Sahrawat (1990) using some Indian soils. LOI was also not correlated with P adsorption maximum in these soils, the organic anions may have blocked the adsorption sites for P adsorption (Gama et al., 1987). In correlation studies Loganathan et al., (1987) reported that adsorption capacities were significantly correlated with percentages of clay and silt, surface area, and different forms of Fe and Al, but not with pH or organic matter. Good correlation between P adsorption and some soil properties like clay content, Fe and Al extracted was reported by Toreu et al., (1988).

The importance of the Freundlich equation is limited by the fact that the constants are not known to describe any particular quantitative or physico-chemical processes

in phosphate adsorption. The other limitation is the adsorption maximum can not be calculated.

The Freundlich equation being one of the oldest equation used, has been shown to give a better fit to phosphate adsorption isotherms in most soils than the more widely used simple Langmuir equation (Fitter and Sutton, 1975; Sibbesen, 1981). Barrow (1978) concluded that the Freundlich equation was the best of the commonly used equations. He further stated that the shape of curves for Freundlich equation was very similar to the Double Langmuir equation. The R^2 values calculated from Freundlich plots were ranged from 87.8 to 99.3 % (Table 4.2). Holford (1982) found that the Freundlich equation gave the best fit to adsorption data, with correlation coefficients of greater than 0.997. Shailaja and Sahrawat (1990) found both Langmuir and Freundlich equations described P adsorption accurately with R^2 values 0.98 and 0.99 respectively at all solution P concentration in soils they studied.

CHAPTER 5

Phosphate adsorption-desorption isotherms

5.1 Introduction

The adsorption and desorption of P in soils are among the key processes governing the availability of phosphorus to plants. Holford (1989) reported that due to the very large variation in the surface area of soil particles
(5-300 m^2g^{-1}), micro-surface reactions (adsorption/ desorption) play dominant roles in controlling the behaviour of phosphorus in soils.

When soluble P is added to a soil, any pre-existing equilibrium is disturbed. There will be a movement of P to the adsorbed form and with the passage of time to the firmly held form. Two types of P adsorption reaction operate over the range of P concentrations found in soils (Ryden et al., 1977a; Ryden and Syers, 1977 and Luscombe et al., 1979). Luscombe et al., (1979) suggested that the first reaction involves a high free energy of adsorption which is characteristic of chemisorption, whereas the second involves a lower free energy of adsorption more like physical type of adsorption. Phosphate adsorbed by the latter mechanism was completely exchangeable with P^{32} during 30 minutes and was reversible with respect to solution P concentration at the same pH (Ryden and Syers,

1977). Olsen and Khasawneh (1980) reported that adsorption occurred at low to medium concentrations of P, whereas at higher concentrations dissolution and precipitation reactions occurred with the formation of new phases.

The phosphate concentration in the soil solution, which reflects the outcome of the reactions between soil and solution phosphate is affected by the release of phosphate from the soil with time (Mendoza et al., 1990). The release of phosphate with time affected both the magnitude and the direction of the change in phosphate concentration in soil solution. They further explained, by assuming adsorption and desorption as reversible, that when the soil is in contact with high levels of phosphate, the dominant effect of time transfers phosphate from the surface of the soil particles towards the interior. As a result, the phosphate is more firmly retained by the soil and the concentration of P in the solution decreases. This prevents movements of phosphate from the interior of soil particles back to the surface. At low levels of phosphate in contact with the soil, the diffusion gradient is in the opposite direction, the effect of time is on the release of phosphate from the surface to the solution. The concentration of phosphate in the soil solution increases. The effect of time has also been reported by Barrow (1989).

It is generally agreed that fast and slow reactions occur during the adsorption (Barrow, 1980). White (1980) suggested that the fast reaction involves only P exchanging for anions at the surface for which the activation energy of exchange is low. The slow reactions may involve many processes for which appreciable activation energy is required, for example the conversion of physically adsorbed to chemisorbed P (Ryden et al, 1977b); the incorporation of P into hydroxy-Al or Fe polymers, or its occlusion in the surface of calcite crystals (Griffin and Jurinak, 1973); or the precipitation of P compounds for which the solubility products have been exceeded (Veith and Sposito, 1977). The slow reaction may also involve P uptake by microbes, which becomes significant within 2-4 hours at 25^{o}C and adsorption of P onto new sites exposed by breakdown of aggregates through shaking (White, 1980). The greater proportion of the P involved in slow reactions is generally held irreversibly (Olsen and Khasawneh, 1980). Syers et al (1970) and Munns and Fox (1976) reported P adsorption to be only partly reversible. However, Barrow and Shaw (1975) argued that reversibility decreased as the adsorption period was prolonged. Furthermore, hysteresis in the adsorption-desorption indicated that phosphate adsorption is not completely reversible (Manikandan and Sastry, 1986), but only a fraction of previously adsorbed P is able to desorb (Barrow, 1979; Stuanes, 1984). White and Taylor (1977) suggested that

the current adsorption of P will be reversible provided the soil is pretreated with soluble P to satisfy the high energy sites. The loosely bound surface P, but not chemisorbed P, was completely desorbed at constant pH and ionic strength (Ryden and Syers, 1977). Barrow (1983c) suggested that phosphate added to soil is slow to desorb and adsorption is reversible in the sense that a continuous curve of adsorbed and desorbed phosphate is obtained when these are measured in opposite directions by increasing or decreasing, the solution of phosphate. He further stated that as the dynamic processes are involved, an earlier position of a plot of adsorbed P against concentration is not retraced when the concentration is changed.

In the laboratory the reversibility of the slow reaction may be studied by reducing the concentration of P in the solution. This may be done by increasing the volume of solution with phosphate free solution or by using anion exchange resin or other P adsorbing material like Fe oxide-coated filter paper (van der Zee et al., 1988). After a specified time the P concentration is again measured and desorption is calculated. There is a general consensus that the reversible adsorption equilibrium may be described by the Langmuir equation or by electrostatic models (Barrow et al., 1981 ; Enfield et al., 1981). However, the assessment of the Langmuir parameters may be complicated by the occurrence of

diffusion-type process (van der Zee et al., 1988). Barrow (1983b) assessed the parameters of both processes by fitting all the model equations simultaneously to the adsorption data by assuming that both processes occur simultaneously and that the diffusion-type process is practically irreversible. Van Der Zee and Van Riemsdijk (1986) estimated the adsorption parameters by fitting model equations to the desorption data and the diffusion-type process was considered to be a precipitation of P. Because the solubility of metal-P precipitates is usually small and dissolution kinetics are often slow, the assumption of irreversibility for the diffusion process was considered acceptable (Van Der Zee et al., 1988).

The desorption process indicates the ability of soil to supply P to plants. Due to the heterogenity of soils many dynamic processes are involved, the desorption behaviour may become quite different to adsorption. Present studies were undertaken to examine the changes involved simultaneously in the adsorption and desorption of phosphate processes.

There was a high degree of experimental error in the initial study of P adsorption-desorption isotherms. The probable sources of these errors were identified in both phosphate adsorption and desorption studies. Theoretical assessment of these errors were carried out and the actual errors which occurred were also measured. Results obtained with the improved techniques showed that the top

end points of adsorption and desorption isotherms with similar P concentration did not meet. To investigate the cause of this discontinuity, experiments on the effect of microbial inhibitors and time of contact on both adsorption and desorption of phosphate were carried out.

5.2 The initial P adsorption/desorption curves

In the initial study of phosphate adsorption and desorption isotherms three soils with different characteristics, Dreghorn No.1 A, Dunlop A and Caprington A were selected from amongst the 14 soils studied earlier.

5.2.1 Materials and methods

In the initial P adsorption experiments 1 g air dried soil was weighed to 2-decimal places into 4 oz glass bottles. 50 cm^3 of solutions containing 1, 5, 10, 20, 30, 40, 50, 70 and 100 µg P cm^{-3} were added to the bottles with a 50 cm^3 pipette. The solutions of different P concentrations were prepared separately from a 1000 µg P cm^{-3} stock solution as already described in Section 2.8.1. The bottles were screw capped tightly and shaken on an end over end shaker for 24 hours. After shaking the soil suspensions were filtered through Whatman No 2 filter papers. The filtrates were stored in a cold room for analysis at the same time as the sample from the P desorption experiment.

The P desorption experiment was carried out simultaneously with the P adsorption experiment. 0.2 g air dried soil was weighed to 2-decimal places in five 4 oz bottles for each soil. 10 cm^3 of 100 µg P cm^{-3} was added into each bottle with a 10 cm^3 pipette. The bottles were marked as 0, 10, 25, 50 and 75 indicating the volume of deionized water in cm^3, to be added after 24 hours shaking. The bottles were screw capped and shaken with P adsorption bottles on an end over end shaker. After shaking for 24 hours, deionized water was added to each bottle according to the volume marked. After adding the water the bottles were again shaken for another 24 hours. After shaking the contents of the bottles were filtered through Whatman No 2 filter papers.

The filtrates obtained from adsorption and desorption experiments were analysed for phosphorus concentration by the method described in Section 2.6., using a Technicon AutoanalyzerII. Sampling rates of 50 and 40 samples per hour were used for the undiluted samples and diluted samples respectively. The concentration of all the different original P solutions was also measured. All phosphorus concentrations in the solutions were calculated from a linear calibration graph. The difference between the amount of phosphorus in the filtrates and the amount of phosphorus in the original solution was used to calculate the amount of P adsorbed by the soil in µgP g^{-1}.

5.2.2 Results and discussion

The phosphorus adsorption and desorption data obtained in this initial study were plotted on one graph for each soil (Fig.5.1 to Fig.5.3) The graphical representation in Dreghorn No.1 A Caprington A and Dunlop A showed that the points were scattered and not following a trend in the adsorption-desorption isotherms. In the adsorption isotherms there were wide gaps between data points in the range of 50 to 100 μg P cm^{-3} equilibrium concentrations which created confusion in drawing a line for the adsorption isotherm. Similarly in the desorption experiment points were very few and wide gaps existed between the points. It was difficult to draw a trend line from these scattered points. In the Dreghorn No.1 A and Caprington A soils there were some negative values on the desorption graph indicating that there was more P in the final equilibrium solution than the P concentration initially added.

Due to the poor shape of adsorption-desorption isotherms from the scattered and limited number of points, it was realised that some experimental errors occurred in the initial study and the experiment needed to be redesigned.

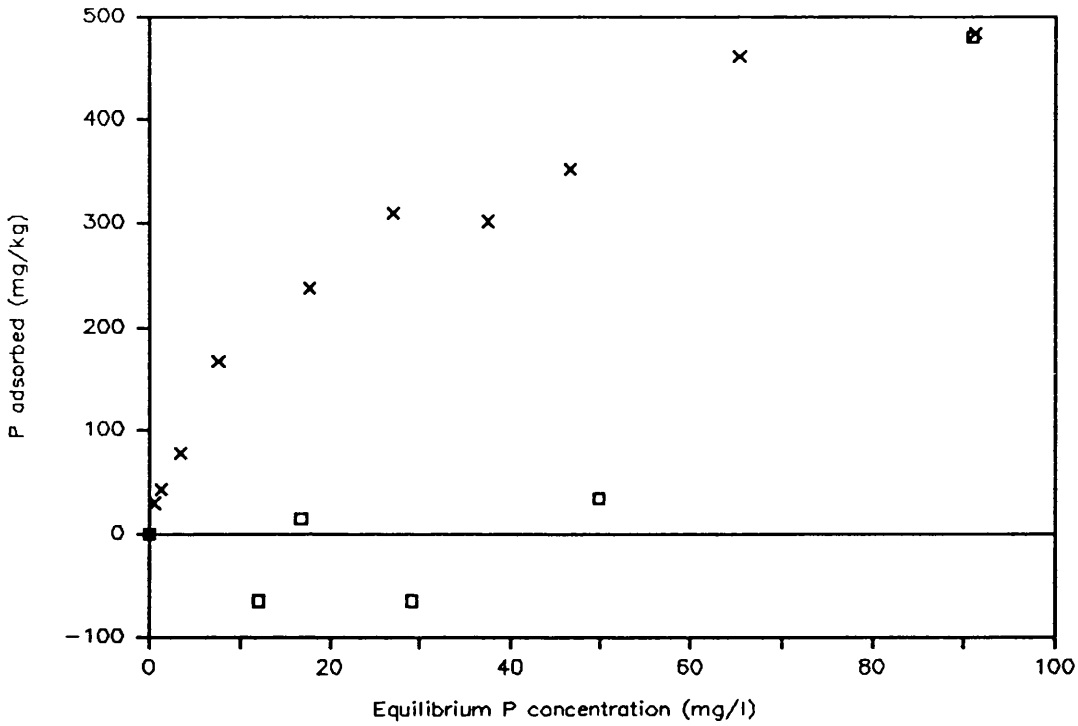


Fig. 5.1 Phosphate adsorption and desorption isotherms for Dreghorn No.1 A soil.

(x) P adsorption

(□) P desorption

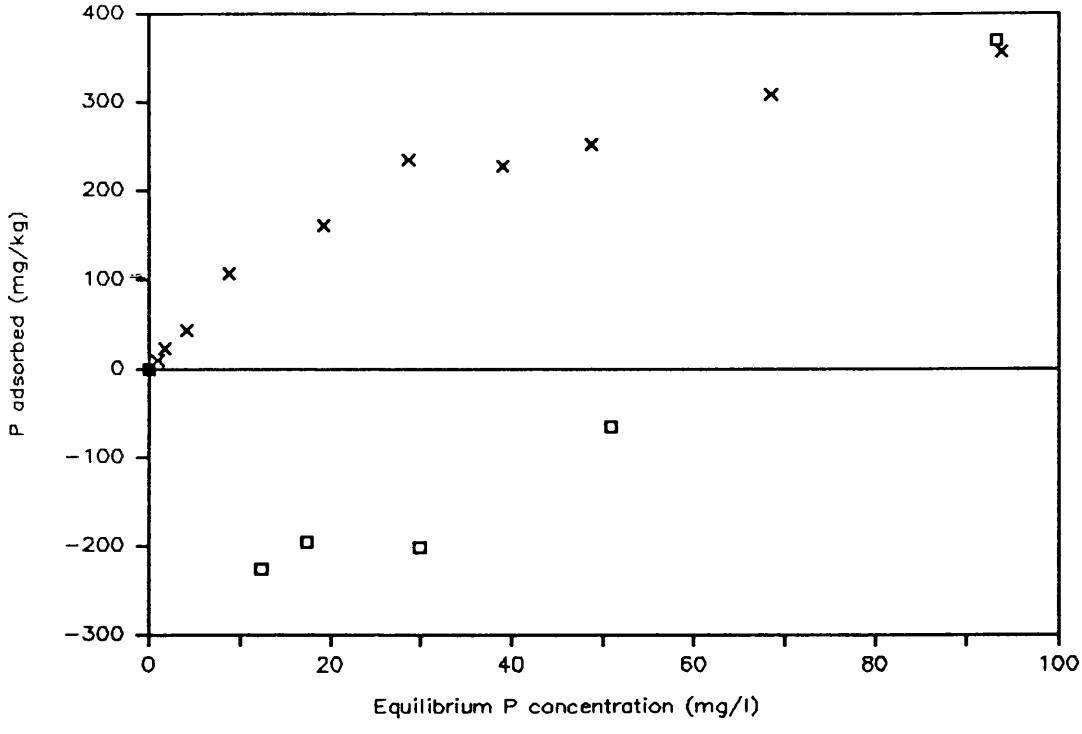


Fig. 5.2 Phosphate adsorption and desorption isotherms for Caprington A soil.

(x) P adsorption

(□) P desorption

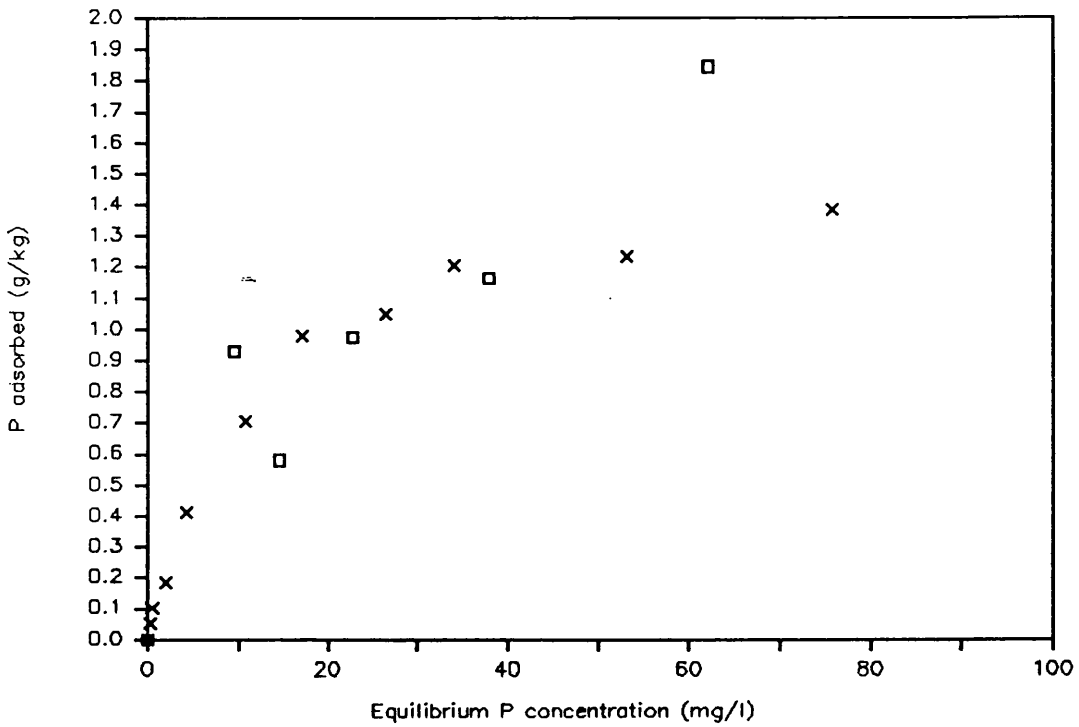


Fig. 5.3 Phosphate adsorption and desorption isotherms for Dunlop A soil.

(x) P adsorption

(□) P desorption

5.3 The analysis of the errors

5.3.1 Introduction

Experimental results are usually subject to errors. For the reliability of final results, the accuracy and precision should be considered. Accuracy may be expressed as how the experimental results closely conform to the actual value. The precision on the other hand may be defined as how well measurements of the same quantity agree with each other. Thus accuracy is the correctness of a measurement whereas precision is the reproducibility of the same measurement.

The errors which affect the experimental results may be of various types: random error or systematic errors. The random errors are caused by irregular experimental technique or measurements. The errors that occur in the execution of an experiment have been regarded as mechanical errors by Gomez and Gomez (1984). These errors may occur in many ways (i) Weighing damp sample rather than dry one. (ii) Weighing a sample still warm. (iii) Contamination during handling. (iv) Imprecise measurements e.g. using 5 cm^3 pipette inadvertently instead of 3 cm^3 pipette. (v) Mismatching units measurement e.g. solutions concentrations expressed as Normal rather than Molar. (vi) Transcription errors (vii) Sample variability errors e.g. soil samples (viii) Instrumental errors such as incorrect calibration, glasswares of different tolerances (ix) Reagent errors

e.g. impure chemicals are used or the solution is made to the wrong concentration. (x) Personal error in handling apparatus e.g. meniscus viewing at different angles, blowing the final drop from a pipette. (xi) Calculating errors e.g. in preparing standard solutions or reagents. (xii) Environmental errors caused due to unknown factors changing without the awareness of the experimenter. Some examples of these errors may be slight variation in temperature, humidity, pressure, light intensity or fluctuations in the flow of electricity main supply.

Some the errors listed above can be eliminated by taking care in experimental technique or by repeating the experiment, while others can be reduced by taking some preventive measures or taking many readings and averaging the results. The most effective prevention of errors is the researcher's awareness of potential errors in relation to the various operations of the experiment.

The following studies were undertaken with the objectives: (i) to examine the various sources of errors and their theoretical assessment; (ii) to measure the actual errors; (iii) to compare errors from theoretical assessment and actual experiment; and (iv) to determine the modifications needed to be made to experimental technique to reduce the errors.

5.3.2 Methods for error measurement

10 μg P cm^{-3} and 100 μg P cm^{-3} standard solutions were prepared from a 1000 μg P cm^{-3} stock solution. For

the measurement of errors due to the Technicon autoanalyzer, analysis of 10 replicates of 10 µg P cm^{-3} solution and 10 replicates of 100 µg P cm^{-3} solution was carried out. P concentration was measured using two calibration standards for each set of measurement of 0 and 10 µg P cm^{-3} and 0 and 100 µg P cm^{-3} standard solutions respectively.

To test the linearity of the calibration curve standard solutions of 0, 5, 10, 15, 20, 30, 40, 50, 60, 70, 80, 90 and 100 µg P cm^{-3}. were prepared from a stock solution of 1000 µg P cm^{-3}. Two standard calibration graphs were made in the range of 0, 5, 10, 15 and 20 µg P cm^{-3} standard solutions and in the range of 0, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 µg P cm^{-3} standard solutions. Each standard solution was analysed in duplicate for each curve and each curve was measured twice.

Three soils Dreghorn No.1 A, Caprington A and Dunlop A were selected for the measurement of the errors in the whole adsorption-desorption experiment. Two points 10 and 100 µg P cm^{-3} in the phosphate adsorption experiment were selected for measuring the errors. 1 g air dried soil was accurately weighed to 4-decimal places into a 4 oz glass bottle. The soil was weighed into two sets of bottles, each set comprising 10 bottles. The weight of soil for each bottle was recorded. 50 cm^3 of 10 or 100 µg P cm^{-3} was added to the respective bottles with two 50 cm^3 bulb

pipettes. The bottles were shaken in an orbital shaker for 24 hours. The temperature of the shaker was kept constant at 30°C. After shaking the contents of bottles were filtered through Whatman No 2 filter papers.

For the measurement of errors in the P desorption experiment two dilutions with 5 and 75 cm^3 of deionized water were selected. For the higher dilution (final soil-solution ratio 1:425) 0.2 g of air dried soil was accurately weighed to 4-decimal places into 4 oz bottles. 10 cm^3 of 100 µg P cm^{-3} solution was added to each bottle with a 10 cm^3 bulb pipette and the bottles were screw capped. For the lower dilution (final soil-solution ratio 1:60) 0.5 g air dried soil was weighed to 4-decimal places into 4 oz bottles. 25 cm^3 of 100 µg P cm^{-3} was added to each bottle with a bulb pipette and the bottle screw capped. There were two sets of bottles in this experiment. Each set of bottles for one dilution point was replicated 10 times. The weight of soil for each bottle was recorded. Both sets of bottles were shaken in the orbital shaker at a constant temperature of 30°C for 24 hours. After the first shaking both sets of bottles were taken out of shaker. 5 cm^3 of deionized water was added with a bulb pipette to each bottle of the lower dilution set and the bottle screw capped again. 75 cm^3 of deionized water was added to each bottle of the higher dilution set. The 75 cm^3 water was added with a 25 cm^3 and a 50 cm^3 bulb pipette. Both sets of bottles were

again shaken for another 24 hours at the same temperature. After the second shaking the contents of bottles were filtered through Whatman No 2 filter papers.

All the filtrates obtained in the P adsorption and desorption experiments were analysed by Technicon Autoanalyzer II for phosphorus concentration. The samples marked with 10 μg P cm^{-3} adsorption and the higher dilution with 75 cm^3 water in the P desorption experiment were analysed using a calibration range 0-20 μg P cm^{-3} with all standard points previously used in the measurement of calibration curve. The samples marked with 100 μg P cm^{-3} adsorption and the lower dilution with 5 cm^3 water in the P desorption experiment were analysed using a calibration range 0-100 μg P cm^{-3} covering all the standard points previously described in the measurement of calibration curve. The sampling rate was reduced to 30 samples per hour to obtain a clear plateau on the peak for each sample. The P concentration in P adsorption and desorption was measured using the relevant calibration range with all standard points.

5.3.3 Results and discussion

5.3.3.1 Theoretical assessment of errors:

In the theoretical assessment of errors the three soils Dreghorn No.1 A, Caprington A and Dunlop A were considered. For the adsorption as well as on desorption of phosphorus two points were selected for comparison.

Keeping in view the procedure adopted in the initial study for phosphate adsorption and desorption, some errors were identified that could adversely affect the results. The following theoretical assessment was based mostly on the experience of the initial study.

1. Calibration

In the initial study the measurement of P concentration was made using linear calibration. The calibration was made using two standard points, zero and a high concentration standard. If an error occurred in either point the whole result was affected. In the calibration curve method the concentration is measured from a curve. The curve is comprised of many points. The error in one point is not likely to affect the measurement. The error in the calibration curve method is clearer. Using two points could lead to a positive or a negative error in the concentration measurement. Deviation above a straight line will also cause a small positive error in the concentration measurement. If the initial concentration is assumed to be correct and is not measured then any deviation from linearity could give a large error in the equilibrium concentration measurement. If both the initial and equilibrium concentrations are measured then the errors will tend to cancel each other. A potential error of 1% of full scale was arbitrary chosen for the theoretical calculation. 0.2 and 1.0 μg P cm^{-3} were assumed to be the errors due to calibration for

the 0-20 μg P cm^{-3} and 0-100 μg P cm^{-3} calibration ranges respectively.

2. Technicon autoanalyzer:

There can be a random error in measurements due to the Technicon autoanalyzer. Such errors are likely to increase with the rate of analysis. Assuming an error of 0.5% of full scale measurement in the calibration ranges 0-20 μg P cm^{-3} and 0-100 μg P cm^{-3} would have errors of 0.1 and 0.5 μg P cm^{-3} respectively.

3. Weighing of soil:

In the first set of adsorption/desorption curve, a 2-digit electronic balance was used for weighing the soil. Due to heterogeneous nature of soil weighing very small amounts could cause an error in the results. In the initial study where the soil was weighed by 2-digit balance the error could occur in the second decimal place. So a lower limit of 0.01 g and a higher limit of 0.05 g error was considered in this case.

4. Volume of phosphate treating solution:

There could be an error due to the measurement of the volume in the P treating solution. The tolerance (systematic error) for a class B 50 cm^3 bulb pipette is ±0.08 cm^3 (BS1583). Adding a small random error, a total error of 0.1 cm^3 was used in calculation.

P adsorption:

In the hypothetical experiment of P adsorption 50 cm^3 of 10 and 100 µg cm^{-3} P solution were added to 1 g of soil. 7 and 90 µg P cm^{-3} were found as the equilibrium concentration in the Dreghorn No.1 A adsorption process the P adsorbed would be 150 µg g^{-1} and 500 µg g^{-1} soil respectively. In Caprington A, for 10 and 100 µg P cm^{-3} initial concentration 8 and 94 µg P cm^{-3} were considered to be the equilibrium concentrations, the P adsorbed would be 100 and 300 µg P g^{-1} respectively. In the same way, for the Dunlop A soil, 3 and 68 µg P cm^{-3} were to be taken as the final equilibrium P concentration the P adsorbed will be 350 µg g^{-1} and 1600 µg g^{-1} soil respectively. The errors assessment hypothesized for Dreghorn No.1 A Caprington A and Dunlop A soils for the P adsorption process is given in Table 5.1.

P desorption:

In the hypothetical experiment of P desorption 25 cm^3 and 10 cm^3 of 100 µg P cm^{-3} were added to 0.5 g and 0.2 g of soil for P adsorption. After 24 h of shaking the solutions were diluted by adding 5 cm^3 and 75 cm^3 deionized water respectively. In Dreghorn No.1 A soil the final solution concentration of P in the former case was found to be 75 µg P cm^{-3} and in latter case 11 µg P cm^{-3} the P adsorbed would be 500 µg P g^{-1} and 325 µg P g^{-1} soil respectively. In Caprington A soil, the final

Table 5.1 Theoretical assessment of errors in P adsorption experiment

Source of error	Calibration range in µg P/cm^3	10 µg P cm^{-3} added		100 µg P cm^{-3} added	
		Error	Error in adsorbed µg P/g soil	Error	Error in adsorbed µg P/g soil
DREGHORN No.1 A:					
Calibration	0-100	1.0 µg P cm^{-3}	50	1.0 µg P cm^{-3}	50
	0-20	0.2 µg P cm^{-3}	10	- P cm^{-3}	-
Technicon autoanalyzer	0-100	0.5 µg P cm^{-3}	25	0.5 µg P cm^{-3}	25
	0-20	0.1 µg P cm^{-3}	5	-	-
Weighing		0.01 g	1.5	0.01 g	5
		0.05 g	7	0.05 g	24
Volume		0.1 cm^3	1	0.1 cm^3	10
CAPRINGTON A:					
Calibration	0-100	1.0 µg P cm^{-3}	50	1.0 µg P cm^{-3}	50
	0-20	0.2 µg P cm^{-3}	10	- P cm^{-3}	-
Technicon autoanalyzer	0-100	0.5 µg P cm^{-3}	25	0.5 µg P cm^{-3}	25
	0-20	0.1 µg P cm^{-3}	5	-	-
Weighing		0.01 g	1	0.01 g	3
		0.05 g	5	0.05 g	14
Volume		0.1 cm^3	1	0.1 cm^3	10
DUNLOP A:					
Calibration	0-100	1.0 µg P cm^{-3}	50	1.0 µg P cm^{-3}	50
	0-20	0.2 µg P cm^{-3}	10	- P cm^{-3}	-
Technicon autoanalyzer	0-100	0.5 µg P cm^{-3}	25	0.5 µg P cm^{-3}	25
	0-20	0.1 µg P cm^{-3}	5	-	-
Weighing		0.01 g	3.5	0.01 g	15.8
		0.05 g	16.7	0.05 g	76.2
Volume		0.1 cm^3	1	0.1 cm^3	10

concentrations of solutions for the above treatments were 78 and 12 μg P cm^{-3} the P adsorbed would 320 and 100 μg P g^{-1} respectively. Similarly in Dunlop A soil the final P solution concentration in lower dilution was 52 μg P cm^{-3} and in higher dilution 9 μg P cm^{-3} the P adsorbed would be 1880 and 1175 μg g^{-1} soil respectively. The estimate of the errors likely to occur in this process are summarized in Table 5.2.

On the basis of the theoretical estimates of errors in P adsorption and desorption phenomena it was observed that the level of error that occurred in desorption was higher than the level of error in the adsorption process except errors due to weighing of the soil. The error in weighing the soil was related to the P adsorbed by each soil. Both in P adsorption and desorption phenomena the higher the P adsorbed in any soil the greater value of the error due to weighing errors. The magnitude of the error in P desorption due to errors in measurement by the Technicon autoanalyzer II and due to calibration were high. Very big errors occurred in the desorption experiment due to the high dilution if samples measured in 0-100 μg P cm^{-3}. The level of errors was lower at high dilution if the P concentration was measured over a low calibration range (0-20 μg P cm^{-3}). Madrid and Posner (1979) and Barrow, Madrid and Posner, (1981) have also pointed out that the errors in P desorption become larger due to large dilutions. The error in P adsorption due

Table 5.2 Theoretical assessment of errors in P desorption experiment

Source of error	Calibration range in µg P/cm^3	Low Dilution (1:50 to 1:60)		High Dilution (1:50 to 1:425)	
		Error	Error in adsorbed µg P/g soil	Error	Error in adsorbed µg P/g soil
DREGHORN No.1 A:					
Calibration	0-100	1.0 µg P cm^{-3}	60	1.0 µg P cm^{-3}	425
	0-20	-	-	0.2 µg P cm^{-3}	85
Technicon autoanalyzer	0-100	0.5 µg P cm^{-3}	30	0.5 µg P cm^{-3}	212.5
	0-20	-	-	0.1 µg	42.5
Weighing		0.01 g	5	0.01 g	15
		0.05 g	24	0.05 g	56
Volume		0.1 cm^3	10	0.1 cm^3	10
CAPRINGTON A:					
Calibration	0-100	1.0 µg P cm^{-3}	60	1.0 µg P cm^{-3}	425
	0-20	-	-	0.2 µg P cm^{-3}	85
Technicon autoanalyzer	0-100	0.5 µg P cm^{-3}	30	0.5 µg P cm^{-3}	212.5
	0-20	-	-	0.1 µg	42.5
Weighing		0.01 g	3	0.01 g	1
		0.05 g	15	0.05 g	5
Volume		0.1 cm^3	10	0.1 cm^3	10
DUNLOP A:					
Calibration	0-100	1.0 µg P cm^{-3}	60	1.0 µg P cm^{-3}	425
	0-20	-	-	0.2 µg P cm^{-3}	85
Technicon autoanalyzer	0-100	0.5 µg P cm^{-3}	30	0.5 µg P cm^{-3}	212.5
	0-20	-	-	0.1 µg	42.5
Weighing		0.01 g	19	0.01 g	12
		0.05 g	89	0.05 g	56
Volume		0.1 cm^3	10	0.1 cm^3	10

to errors in volume was associated with the concentration of phosphate treating solution. The higher the concentration of P the greater the magnitude of the error.

It may be concluded from the hypothetical study of error analysis that the errors in desorption experiment were greater than in the adsorption experiment. The errors were related primarily to errors in the P concentration measurement and the level of error increased with high dilutions. The errors in weighing the soil increased as the amounts of P adsorbed by the soil increased.

5.3.3.2 Measurement of experimental errors:

Errors due to Technicon autoanalyzer:

To quantify the errors in the P adsorption-desorption experiment and to measure the reproducibility of the analysis by the Technicon autoanalyzer a series of experiments were run each with 10 replicates. The experiments were based on the initial theoretical assessment of errors. The P concentrations were measured by Technicon autoanalyzer and the data were statistically analysed. The errors were quantified by standard deviation and confidence interval. Standard deviation is one of the best way of measuring the variability in results and it is often useful to derive confidence

limits within which true result likely to fall(Lee and Lee, 1983).

For the replicated analysis of 10 µg P cm^{-3} and 100 µg P cm^{-3} standard solutions the means, standard deviations and 95% confidence intervals are presented in Table 5.3.

Table 5.3 Variability in P concentration in 10 replicated measurements of standard P solutions

Sample No.	P concentration measured (µg P cm^{-3})	
	10 µg P cm^{-3}	100 µg P cm^{-3}
1	9.94	100.52
2	10.08	100.39
3	9.98	100.32
4	10.00	100.40
5	9.95	100.43
6	9.99	100.48
7	10.02	100.86
8	10.03	100.33
9	10.02	100.48
10	10.09	100.71
Mean	10.01	100.49
Standard deviation	0.049	0.171
95% Confidence interval	9.97-10.04	100.4-100.6

The results showed that the standard deviations for 10 µg P cm^{-3} and 100 µg P cm^{-3} were about 0.5% and 0.2% of the mean respectively. The 95% confidence intervals

for 10 µg P/cm^3 and 100 µg P cm^{-3} were found to be ±0.3% and ±0.1% of the mean respectively. The errors associated with the analysis of the P solutions due to the Technicon autoanalyzer were low compared to the earlier theoretical estimate of errors (Table 5.1 and 5.2). The variability associated with the Technicon autoanalyzer was also measured by Mazumder (1992). He found a standard deviation of 0.2% of the mean in the replicated analyses of 1 µg/cm^3 ammonium-N and nitrate-N solutions.

Errors due to calibration curve:

Both calibration ranges 0-20 µg P cm^{-3} and 0-100 µg P cm^{-3} showed very good fit to a straight line (Fig.5.4 and Fig.5.5). The R^2 values in both calibration ranges were found to be 100%. The data was also fitted to a quadratic equation. A comparison between the linear and quadratic equations was made in terms of the residual sum of squares for each best fit line. The results are shown in Table 5.4.

Table 5.4 Comparison of goodness of fit of calibration data to quadratic and linear equations

Calibration range (µg P cm^{-3})	Residual sum of squares	
	Linear	Quadratic
0-100	0.49	0.49
0-100	1.59	1.59
0-20	0.10	0.07
0-20	0.24	0.24

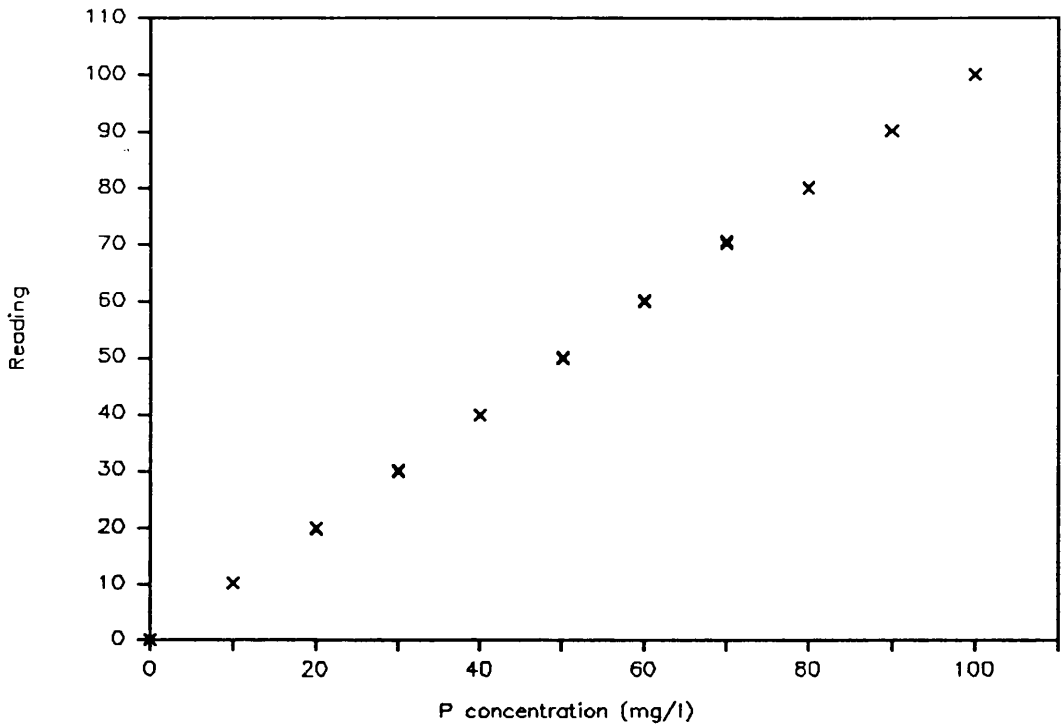


Fig. 5.4 Calibration of standards 0-100 mg P/l

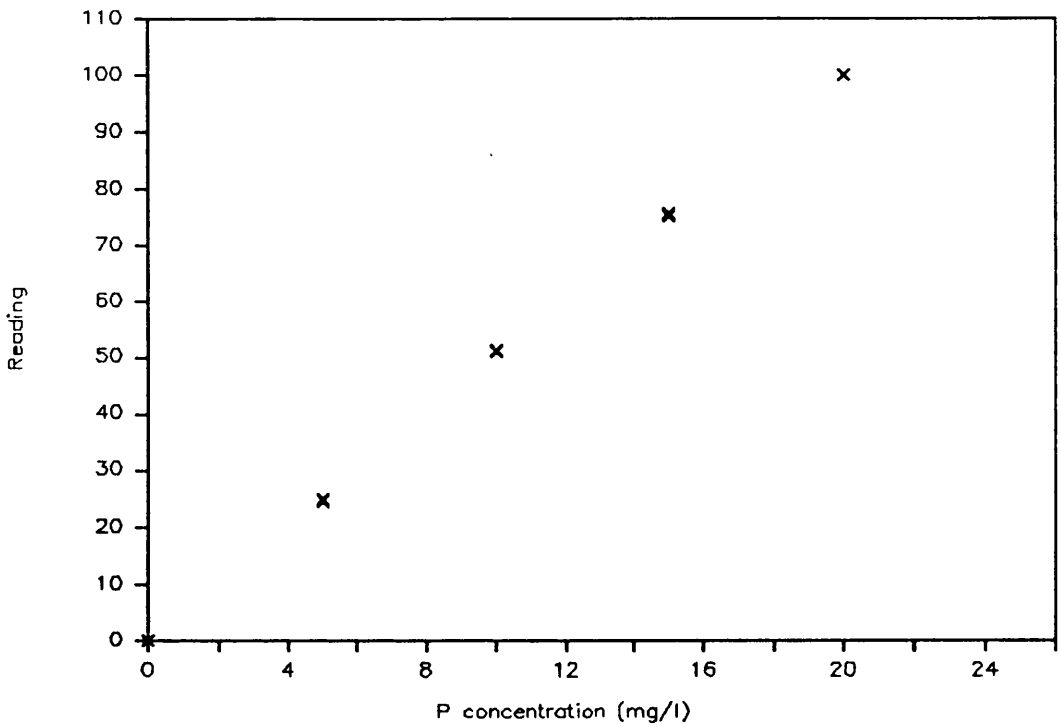


Fig. 5.5 Calibration of standards 0-20 mg P/l

The very low values of residual sum of squares indicated that both the equations were a very good fit to the data. There was very little difference between the fits to linear and quadratic equations confirming that the calibration was linear not curved.

Errors in the whole experiment:

The errors measured in 10 replicates of the two points on the adsorption and the two points on the desorption curves for each of the 3 soils are presented in Table 5.5. The data in Table 5.5 showed that the standard deviation was in the range 0.2-1.0% of the means. This corresponds to a range of ±0.2-1.3% of the means (95% confidence interval). The results also showed that the variability in the P desorption was similar to the variability in the P adsorption measurements. In the whole experiment the errors were found to be very much less than the errors suggested in the theoretical estimate. The errors in general have been reduced by adopting certain measures: (1) The experiment was replicated to reduce the experimental error due to soil heterogenity (Gomez and Gomez, 1984). (2) The P concentration was measured on a linear calibration graph using many points rather than 2 points on linear basis. (3) The analysis rate was reduced from 40 to 30 samples per hour, to get more reproducibility from Technicon Autoanalyzer when using the automated dilution system. (4) To reduce the risk of error

Table 5.5 Measured errors in P adsorption and desorption experiments

Treatments	Equilibrium P concentration (μg P cm^{-3})		
	Mean	Standard deviation	95% Confidence interval
DREGHORN No.1 A			
10 μg P cm^{-3} adsorption	7.17	0.075	7.1 - 7.2
100 μg P cm^{-3} adsorption	90.5	0.198	90.3 - 90.6
P desorption 1:60 dilution	74.11	0.357	73.8 - 74.4
P desorption 1:425 dilution	10.83	0.061	10.8 - 10.9
CAPRINGTON A			
10 μg P cm^{-3} adsorption	8.2	0.074	8.2 - 8.3
100 μg P cm^{-3} adsorption	93.8	0.303	93.6 - 94.0
P desorption 1:60 dilution	78.3	0.310	78.1 - 78.5
P desorption 1:425 dilution	11.46	0.033	11.4 - 11.5
DUNLOP A			
10 μg P cm^{-3} adsorption	3.62	0.046	3.6 - 3.7
100 μg P cm^{-3} adsorption	68.8	0.329	68.5 - 69.0
P desorption 1:60 dilution	52.15	0.464	51.7 - 52.5
P desorption 1:425 dilution	8.66	0.10	8.6 - 8.7

in weighing a 4-digits electronic balance was used instead of a 2-digit balance. (5) To minimize the error from measuring volume, glass bulb pipettes (which have relatively less tolerance than other volume measuring vessels) were used for all measurement of volume. Grava (1975) has described inconsistencies in P test results due to variations in adopting laboratory procedures.

5.4 The P adsorption and desorption isotherms

5.4.1 Introduction

As a result of the analysis of errors, some modifications were made in the experimental technique as suggested in the preceding paragraph. To obtain curves for P adsorption and desorption the same three soils were selected as used previously. These soils were Dreghorn No.1 A, Caprington A and Dunlop A. These soils are acidic in nature, the site descriptions and other physico-chemical characteristics of these soils have already been discussed in chapter 2.

5.4.2 Materials and Methods

For the P adsorption experiment 15 phosphorus concentrations were included instead of the 10 used previously. The phosphorus concentrations used were 0.2, 0.5, 1.0, 2.0, 5.0, 10.0, 20.0, 30.0, 40.0, 50.0, 60.0, 70.0, 80.0, 90.0 and 100.0 μg P cm^{-3}. All the various phosphorus concentration solutions were prepared from a

1000 μg P cm^{-3} stock solution in 500 cm^3 volumetric flasks. In this experiment a more careful account of the weight of soil was taken. The weight of soil was taken to 4 digits instead of 2 digits by using a more sensitive electronic balance. In the P adsorption experiment 1 g air-dried 2 mm sieved soil, was weighed into the 4 oz glass bottles. The soil in each bottle was treated with 50 cm^3 of the respective standard P solution.

In the P desorption studies either 0.2 g or 0.5 g air dried 2 mm sieved soil was weighed with 4-digit balance into 4 oz glass bottles. Either 10 cm^3 or 25 cm^3 of 100 μg P cm^{-3} solution were added to the bottles, maintaining a 1:50 soil-solution ratio.

All the bottles prepared in the adsorption and desorption studies were in duplicate. The bottles marked for P desorption were shaken along with the P adsorption bottles in an orbital shaker at constant temperature of 30°C. After shaking for 24 hours, all the bottles were taken off the shaker. The soil suspensions in P adsorption bottles were filtered through Whatman No 2 filter papers into polythene bottles. The filtrates were stored in a cold room for P analysis later with the samples from the P desorption experiment.

The P desorption bottles, marked for 0.5 g soil, were treated with 0, 3, 5, 10, 15, 20, 25, 35 or 50 cm^3 deionized water, while the bottles marked for 0.2 g soil,

were treated with 25, 50 or 75 cm^3 deionized water. The volume of deionized water for each treatment was measured by the respective glass bulb pipette. All the P desorption bottles were shaken for another 24 hours in the same orbital shaker at the same temperature. After shaking the soil suspensions were filtered through Whatman No 2 filter papers into polythene bottles.

All the filtrates were analysed for phosphorus concentration by the Technicon Autoanalyzer. The analysis rate was reduced from 40 or 50 to 30 samples per hour. To measure the phosphorus concentration the samples were split into three batches. The samples which could come in the range 0-5 µg P cm^{-3} were measured on a linear basis with two standards of 0 and 5 µg P cm^{-3} . The samples in the range 5-20 µg P cm^{-3} were measured using a best fit linear calibration with standards of 0, 5, 10, 15 and 20 µg P cm^{-3}. The samples in 20-100 µg P cm^{-3} were measured using a best fit linear calibration with standards of 0, 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 µg P cm^{-3}. The P concentration in the original solutions was also measured. The difference between the amount of P added and the amount in the filtrate was taken as the amount of P adsorbed by the soils from solution.

5.4.3. Results and discussion

Phosphate adsorption and desorption isotherms derived from subsamples of the three soils studied are presented on a uniform scale in Fig. 5.6 to 5.8. By

plotting the adsorbed P against the equilibrium P in solution, it was observed the points fell on a smooth curve and showed little scatter. The adsorption isotherms in all the three soils were greatly improved compared to the first set of curves (Fig. 5.1-5.3). The desorption isotherms were found to be more or less similar in Dreghorn No.1 A and Dunlop A soils. Some points on the desorption isotherm of Caprington A were more scattered. All the desorption isotherms occurred above the adsorption isotherms. The slope of the desorption lines appears smaller than the slope of the adsorption isotherms. This, and the consequent wide gap at high dilutions, indicates that more phosphate was adsorbed than was later desorbed, as other workers found with kaolinite, gibbsite, and pseudoboehmite (Muljadi et al., 1966; Kafkafi et al., 1967). It may be either due to the penetration of phosphate into the soil particles (Barrow, 1983a and 1989) or due to precipitation (Barrow, 1974; Barrow and Shaw, 1975; Manikandan and Sastry, 1986 ; Van Der Zee et al., 1988). The results are consistent with the studies of Brewster et al. (1975) and Le Mare (1981) who found that soils desorbed less phosphate than they had adsorbed.

The adsorption and desorption lines did not meet at the point from where dilutions were made as they should. It was thought that there could be two possibilities for this difference. Either P continued to be adsorbed or

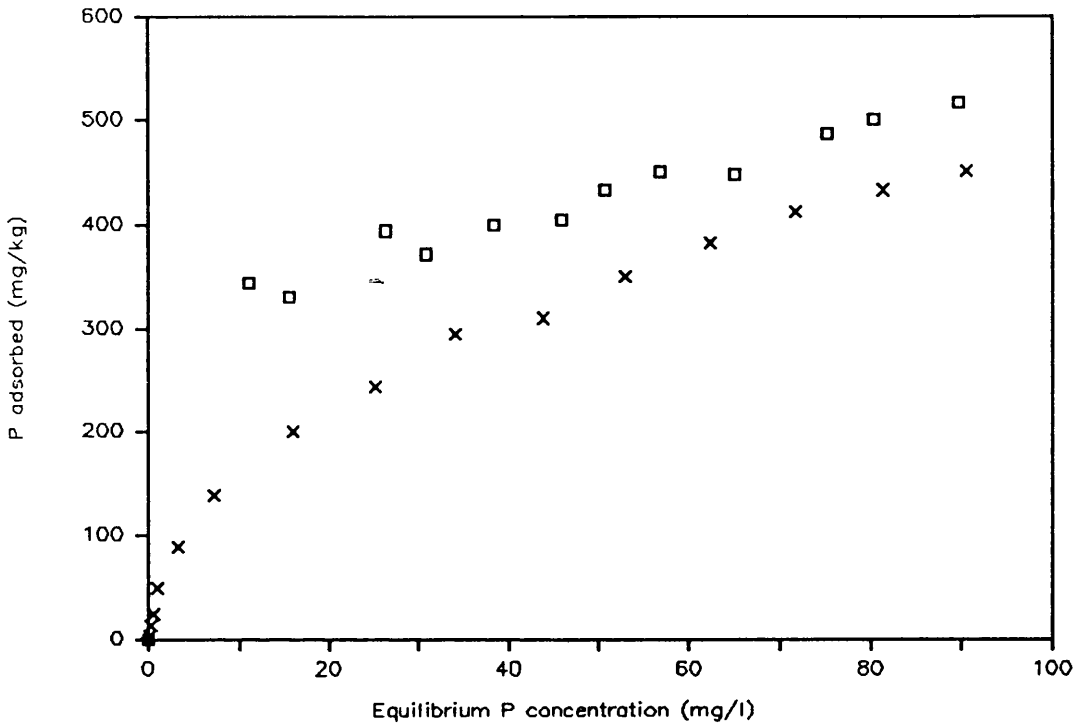


Fig. 5.6 Phosphate adsorption and desorption isotherms for Dreghorn No.1 A soil.

(x) P adsorption

(□) P desorption

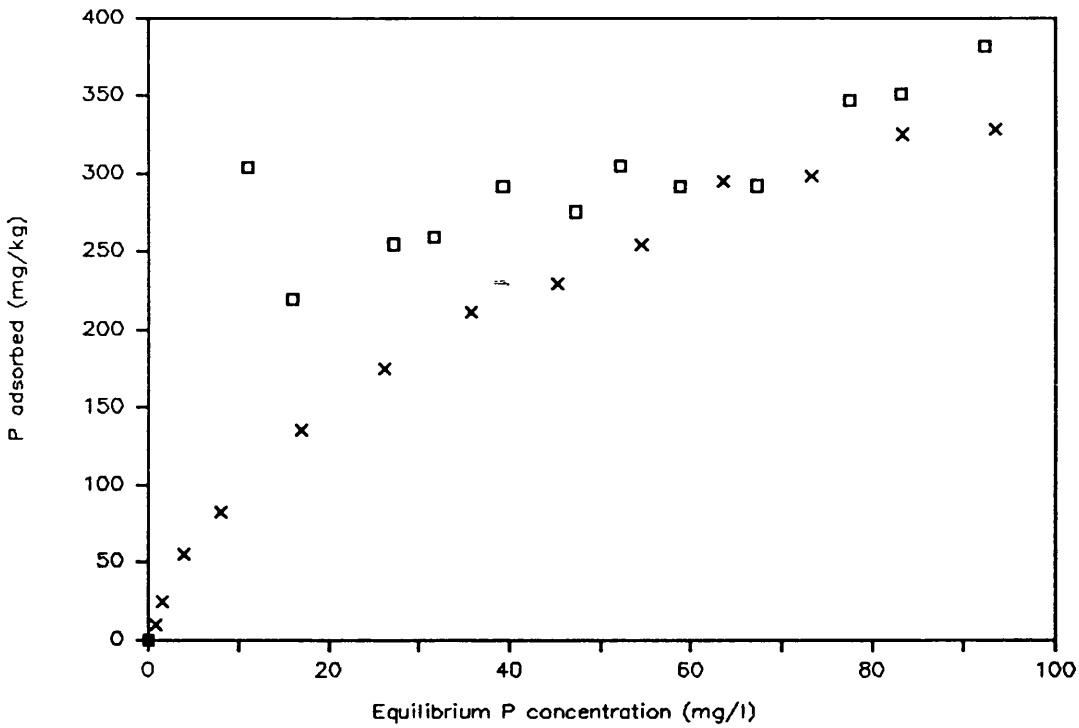


Fig. 5.7 Phosphate adsorption and desorption isotherms for Caprington A soil.

(x) P adsorption

(□) P desorption

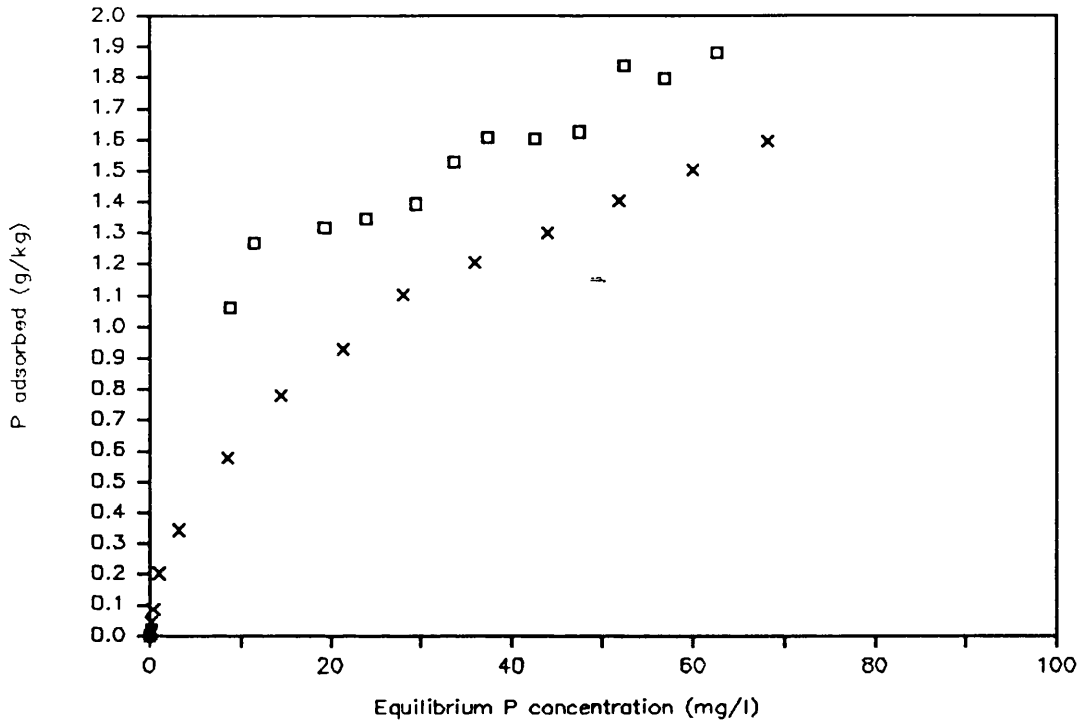


Fig. 5.8 Phosphate adsorption and desorption isotherms for Dunlop A soil.

(x) P adsorption

(□) P desorption

microbial uptake of P had taken place during the additional time of shaking in the desorption experiment. White (1980) reported that there could be microbial P uptake with passage of time. To find the cause of the gap between the two end points of adsorption and desorption curves, further study was carried out.

5.5. Effect of time of shaking on adsorption and desorption

5.5.1 Introduction

The concentration of phosphorus in the solution phase varies greatly with time due to a complex series of adsorption-desorption reactions which control the transfer of phosphorus between adsorbed and solution phases. Many research workers have reported the effect of time on the fate of phosphorus in soil solution (Barrow and Shaw, 1975; Fiskell et al., 1979; Mendoza et al., 1990). The magnitude of P adsorption increased with period of mixing (Olsen and Watanabe, 1957). Phosphate adsorption by soils is usually rapid at first, but then slows with time (Munns and Fox, 1976; Ryden et al., 1977b; Barrow et al., 1981; Van Der Zee and Van Riemsdijk, 1986; Gama et al., 1987; Torrent, 1987). Gama et al. (1987) reported that the adsorption was high during the first half hour and slowed down with time. The mechanism of the slow reaction in soil is further obscured by microbial uptake of P and exposure of new reactive sites (White, 1980).

At a given P concentration, logarithmic plots of adsorption versus time were approximately linear (Barrow, 1983a). He also observed that the desorption of P was faster, when the period of prior contact between soil and phosphate was shorter and initial desorption was followed by a resumption of adsorption.

The objective of this study was to find the effect of different intervals of time on P adsorption and desorption.

5.5.2 Materials and Methods

Three soils Dreghorn No.1 A, Caprington A and Dunlop A were used in this study. From the previous studies on P adsorption and P desorption, two points were selected on both curves. To determine the effect of time of shaking on P adsorption approximately 1.0000 g of air dried soil was weighed accurately into 3 replicated 4 oz screw capped glass bottles for each treatment. 50 cm^3 of 5 or 100 µg P cm^{-3} solutions were added to the bottles. The bottles were shaken on orbital shaker for 6, 12, 24, 48 and 72 hours at a constant temperature of 30^oC. The soil suspensions were filtered through Whatman No 2 filter papers. The P concentration was measured colorimetrically by Technicon Autoanalyzer II according to the method described in Section 5.4.2.

In the desorption study, approximately 0.5000 g sample of air dried soil was weighed accurately into

replicated 4 oz glass bottles. The soil sample in each bottle was treated with 25 cm^3 of 100 µg P cm^{-3} solution. All the bottles were shaken on an orbital shaker at a constant temperature of 30°C. After 24 hours of continuous shaking, the bottles for each soil were divided into 3 treatments each replicated 3 times. The first treatment was filtered immediately without dilution. The remaining bottles were treated with either 5 cm^3 or 50 cm^3 of deionized water and shaken for 0, 0.5, 1, 2, 4 and 6 hours. After shaking for the appropriate time the samples were filtered immediately through Whatman No 2 filter papers. The P concentration in each treatment was measured according to procedure given in Section 5.4.2.

5.5.3 Results and discussion:

The time dependency of phosphate adsorption and desorption in soils is particularly important to the efficiency with which plant roots take up P from the soil solution. The effects of time of contact between soil and P solutions on P adsorption as well as the time effects on P desorption are shown in Tables 5.6 to 5.8, which have been further illustrated in figures 5.9 to 5.12.

In the P adsorption studies very highly significant differences ($P < 0.001$) were observed at both P concentrations tested due to shaking time (Tables 5.6 - 5.6a and Fig. 5.9 - 5.10). It was observed from the data that the P concentration in soil solution decreased with

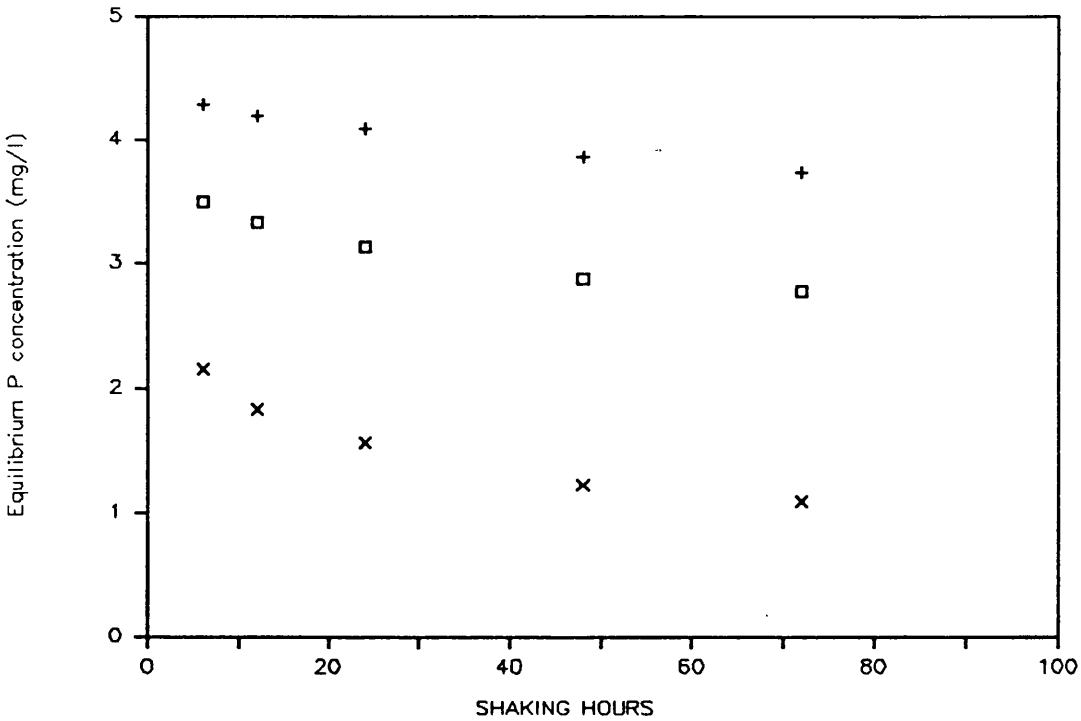


Fig. 5.9 Effect of different times of shaking on adsorption of P in 5 µg P cm^{-3} solution.

(□) Dreghorn No.1 A

(+) Caprington A

(x) Dunlop A

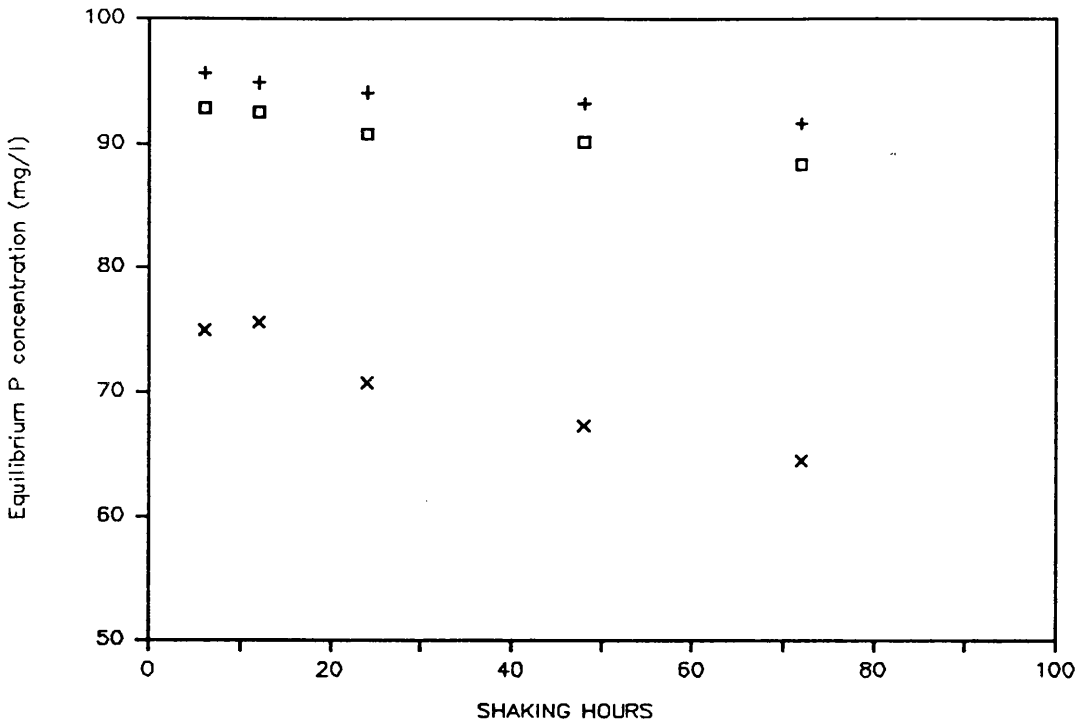


Fig. 5.10 Effect of different times of shaking on adsorption of P in 100 µg P cm^{-3} solution.

(□) Dreghorn No.1 A

(+) Caprington A

(x) Dunlop A

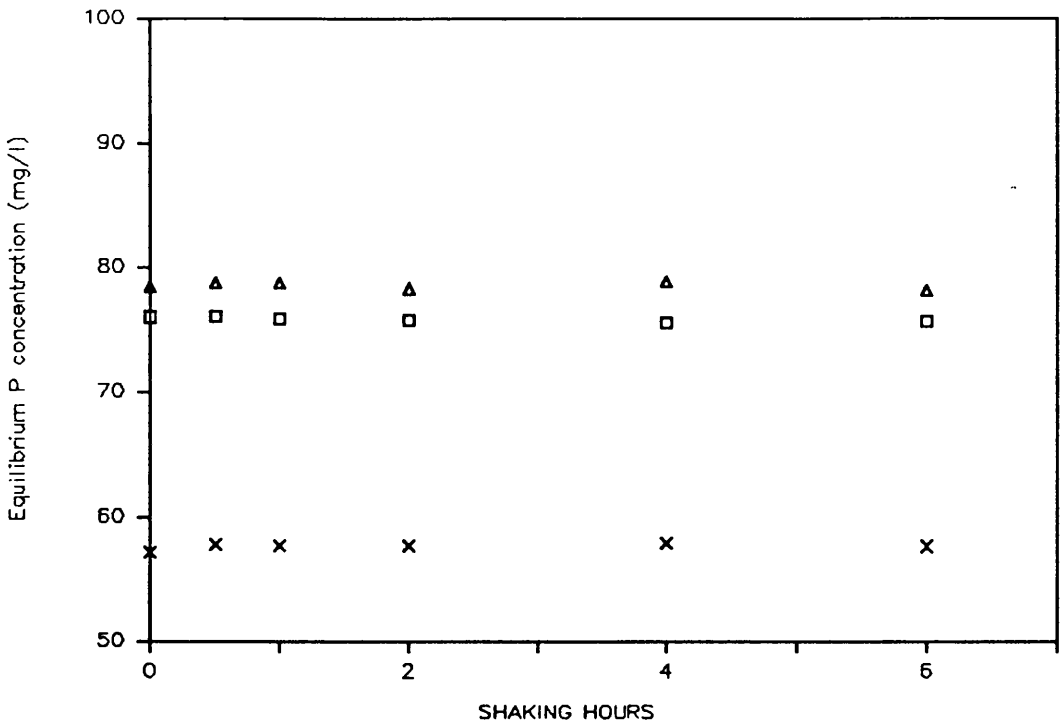


Fig.5.11 Effect of different times of shaking on P desorption after shaking with 100 µg P cm^{-3} for 24 hours followed by dilution with water from a soil:solution ratio of 1:50 to 1:60.

(□) Dreghorn No.1 A

(△) Caprington A

(x) Dunlop A

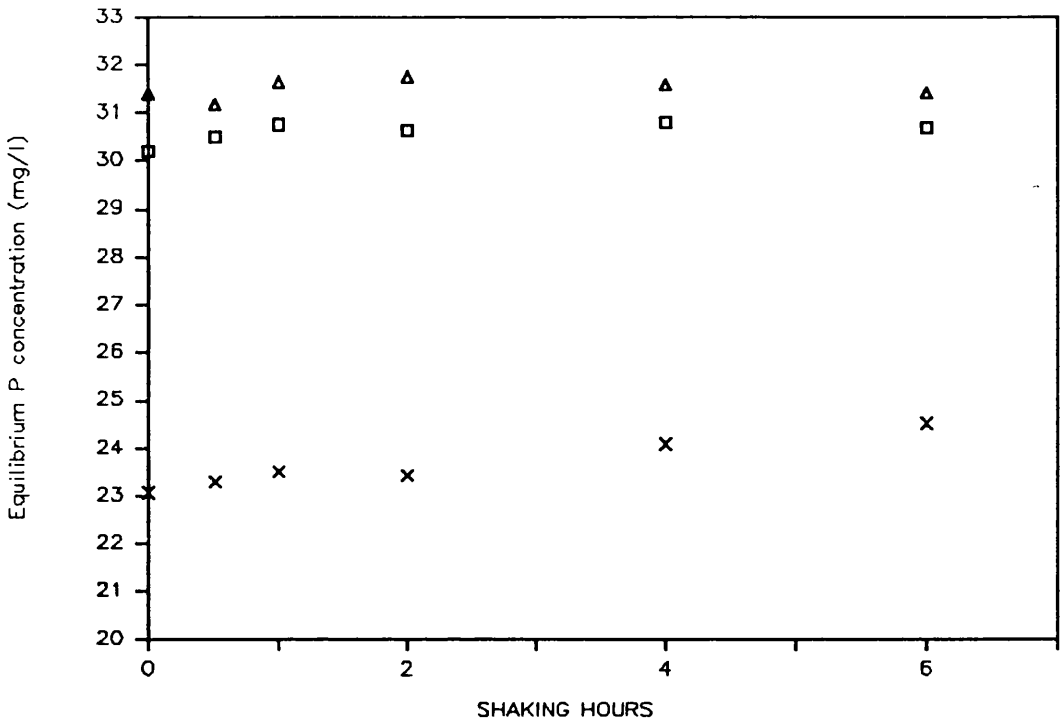


Fig. 5.12 Effect of different times of shaking on P desorption after shaking with 100 µg P cm^{-3} for 24 hours followed by dilution with water from a soil:solution ratio of 1:50 to 1:150.

(□) Dreghorn No.1 A

(△) Caprington A

(x) Dunlop A

Table 5.6 Effect of time of shaking on P adsorption in soils treated with 5 µg P cm^{-3} solution

Shaking time (hours)	Equilibrium P concentration (µg P cm^{-3})		
	DREGHORN No 1 A	CAPRINGTON A	DUNLOP A
6	3.50	4.29	2.16
	***	**	***
12	3.34	4.19	1.84
	***	**	***
24	3.14	4.09	1.57
	***	***	***
48	2.88	3.86	1.23
	**	**	*
72	2.78	3.74	1.09

*=Significant difference in two consecutive means at 0.05
**=Significant difference in two consecutive means at 0.01
***=Significant difference in two consecutive means at 0.001

Table 5.6a Effect of time of shaking on P adsorption in soils treated with 100 µg P cm^{-3} solution

Shaking time (hours)	Equilibrium P concentration (µg P cm^{-3})		
	DREGHORN No 1 A	CAPRINGTON A	DUNLOP A
6	92.87	95.68	74.99
	NS	**	NS
12	92.58	94.89	75.58
	***	**	***
24	90.83	94.17	70.76
	**	***	***
48	90.18	93.27	67.32
	***	***	***
72	88.29	91.59	64.52

NS=No significant difference in two consecutive means at 0.05
*=Significant difference in two consecutive means at 0.05
**=Significant difference in two consecutive means at 0.01
***=Significant difference in two consecutive means at 0.001

increasing time of shaking in all the soils. Similar observations were also recorded by Olsen and Khasawneh (1980); Barrow (1983a) and Kato and Owa (1989) who reported that P adsorption increased with the increasing time. However, the difference was less conspicuous at the lower P concentration. This may be due to the reason that much of the P has been strongly adsorbed in all the soils, whereas at the higher concentrations the difference in behaviour may be due to different adsorption capacities of the soils. Gama et al., (1987) observed that the effect of period of mixing on P adsorption in the soils was more pronounced at 50 than at 5 μg P cm^{-1} initial P concentration. Mendoza et al. (1990) observed that changes with time of the phosphate concentration in solution differed between soils and the magnitude and direction of the changes were affected by the level of addition of phosphate. At high level of addition, the concentration of phosphate in solution decreased with time.

In this study the highest reduction in P concentration in all the soils was observed before the first measurement at six hours. This indicated that the reaction was at first rapid and then slowed down with time. Similar observations have been recorded by many research workers (Imai et al., 1981; Bolan et al., 1985; Gama et al., 1987 and Barrow, 1989). Various workers have studied the rates of P adsorption process in soils, such

studies have measured the rate of P adsorption over intervals of minutes, hours or days. Oskay (1986) found that 72 to 93.2 % of added phosphate was adsorbed after one hour and there was a rapid decline in equilibrium P concentration due to fast adsorption of P by soils. He also observed that the amount of adsorbed P continued to increase at a relatively lower rate by the extension of equilibrium period. Kato and Owa (1989) also observed that P adsorption rate was extremely high within the first 2 minutes, and decreased rapidly with the equilibration time. Van Der Zee and Van Riemsdijk (1988) suggested that overall reaction of phosphorus with soil is the result of two processes, a relatively fast, reversible adsorption and a relatively slow, practically irreversible, diffusion-limited precipitation. The initial fast reaction is completed within 1-3 hours (Fitter and Sutton, 1975).

The effects of different times of shaking on P desorption after a low dilution as well as after a high dilution are presented in Tables 5.7-5.8 and further illustrated in Fig. 5.11- 5.12.

The theoretically calculated P concentration due to simple dilution was compared with the measured equilibrium P concentration immediately after dilution. It was observed that there was no significant instantaneous P desorption either after low dilution or

Table 5.7 Effect of shaking time on P desorption in soils diluted from a soil:solution ratio of 1:50 to 1:60 with deionized water

Shaking time (hours)	Final equilibrium P concentration (μg P cm^{-3})		
	DREGHORN No 1 A	CAPRINGTON A	DUNLOP A
0^+	76.10	78.31	56.90
0	76.08	78.48	57.16
0.5	76.16	78.79	57.84
1	75.95	78.85	57.72
2	75.83	78.33	57.74
4	75.55	78.92	57.95
6	75.72	78.19	57.67
Instantaneous P desorption	NS	NS	NS
Trend with time	* (Adsorption)	NS	NS

+ Theoretically calculated P concentration due to dilution
NS Not significantly different at 0.05 level of probability
* Significantly different at 0.05 level of probability

Table 5.8 Effect of different shaking hours on P desorption in soils diluted from 1:50 to 1:150

Shaking time (hours)	Final equilibrium P concentration (μg P cm^{-3})		
	DREGHORN No 1 A	CAPRINGTON A	DUNLOP A
0^+	30.44	31.33	22.76
0	30.21	31.40	23.07
0.5	30.50	31.37	23.29
1	30.76	31.64	23.52
2	30.63	31.75	23.43
4	30.80	31.58	24.09
6	30.70	31.41	24.52
Instantaneous P desorption	NS	NS	NS
Trend with time	NS	NS	*** (Desorption)

+ Theoretically calculated P concentration due to dilution
NS Not significantly different at 0.05 level of probability
*** Significantly different at 0.001 level of probability

after high dilution. From the regression analyses of the data, it was observed that the trend with time was not significant in Caprington A and Dunlop A soils after low dilution and Dreghorn No 1 A and Caprington A soils after high dilution. The trend was significant in Dreghorn No 1 A soil after low dilution and very highly significant in Dunlop A soil after high dilution. The different behaviour in different soils may be due to their different P adsorption capacities. The significant trend with time after low dilution in Dreghorn No 1 A soil was toward adsorption. On the other hand in Dunlop A soil a very highly significant trend was toward P desorption. In this study there was significant P desorption only in Dunlop A soil, whereas in the other soils there was no evidence of P desorption. The results are consistent with the observations of Madrid and Posner (1979) and Madrid and Arambarri (1985) who found readsorption of P in desorption after 1 day in some soils and two iron oxides respectively. Many workers have reported that the extent of P reversibility decreased as the period is prolonged (Barrow and Shaw, 1975; Barrow, 1983b). However, Madrid and Posner (1979) suggested that extremely high dilution is required to desorb P completely.

From the present study it may be concluded that the amount of phosphorus adsorbed to the soils did not desorb completely. Either fraction of added P was further

adsorbed by the soils or utilized by microbial uptake of P.

5.6 Soil sterilization

5.6.1 Introduction

From the studies in preceding sections, it was observed that the P adsorption and desorption curves did not meet at the point where dilutions were made (Section 5.4.3, Fig. 5.5 to 5.7). To find the cause of the gap between the two end points detailed study on effects of time of shaking on P adsorption and desorption was carried out. It was observed that P adsorption significantly increased with increasing times of shaking (Tables 5.6-5.6a and Figs. 5.9-5.10). In the P desorption study, there was no evidence of the reversibility of P uptake with time of shaking except in Dunlop A soil (Tables 5.7-5.8 and Figs. 5.11-5.12). It was thought that either some of the P may have been converted to microbial biomass or further P adsorption has occurred, resulting in P become non-labile. In order to distinguish between chemical and biological processes, soil sterilization was carried out to avoid microbial activity so that the effect of the chemical changes becomes evident.

Many techniques have been used for sterilizing the soil such as autoclaving, irradiation and using chemicals. Autoclaving or fumigation have the advantage that soil can be sterilized in the experimental

containers on site whereas soil sterilized by gamma irradiation has usually to be sent in sealed containers to the radiation source and subsequently transferred aseptically to experimental vessels. Autoclaving (121^{o}C) for 1 hour and gamma irradiation (3.0 megarads) were compared by Salonius et al., (1967). Both treatments induced certain changes in soil. Radiation has less effect on soluble organic matter and total water extractable electrolytes than autoclaving. Cline et al., (1985) reported that soil sterilization by irradiation had no effect on sodium bicarbonate extractable-P and water extractable-P levels, compared to non-sterilized soil. They further observed that soil sterilization by autoclaving had a much greater effect on indigenous soil P than irradiation.

Since Birch (1961) was the first to propose the use of chloroform ($CHCl_3$) as biocidal agent, a number of workers have utilized $CHCl_3$ fumigation to estimate the soil microbial biomass P (Cole et al., 1978; Chauhan et al., 1979; Stewart et al., 1980; William and Sparling, 1984). Microbial P released by the $CHCl_3$ treatment was adsorbed by soil during fumigation and extraction, in the methods proposed by Brookes et al., (1982) and Hedley and Stewart (1982), it was extracted by 0.5M $NaHCO_3$ (pH 8.5). It was assumed that only microbial P was released by $CHCl_3$ treatment. McLaughlin et al., (1986) measured phosphorus in the soil microbial biomass in field soils,

using a range of biocides in combination with various extractants. They found chloroform and hexanol as the most effective biocides. They suggested that soil should be treated with biocide or extracted immediately after sampling to avoid quantitative or qualitative changes in the biomass. They also tested three methods to correct for adsorption of released inorganic-P (P_i). In one method 20 µg P g^{-1} soil was added and the percentage recovery of added P determined (Brookes et al., 1982). A range of P additions (0.3-20.0 µg P g^{-1} soil) was used in the second method and a adsorption isotherm was constructed. The third method involved isotopic dilution. To minimize the microbial uptake of P in control soils, a 2 h shaking was used and the isotope was added after 1 h shaking. But attempts to correct for adsorption of released $^{31}P_i$ using ^{32}P dilution were unsuccessful. It is evident from the preceding discussion that chloroform has been used extensively as an effective soil sterilant.

Skipper and Westermann (1973) studied the comparative effects of various soil sterilization procedures. They found that sodium azide inhibited microbial populations in soil. Some other workers have also reported the biocidal activity of sodium azide in soil (Kelley and Rodriguiz-Kabana, 1979; Rozycki and Bartha, 1981 and Wolf et al., 1989). Wolf et al. (1989) investigated effectiveness of sterilization of three soils with dry heat, Cobalt-60 irradiation, propylene

oxide, mercuric chloride, autoclaving, sodium azide, microwaves, chloroform and antibiotics. They found that all the various treatments tested were effective soil sterilization procedures, but resulted in substantial physical and chemical changes in soils except mercuric chloride treatment. Mercuric chloride is highly toxic to human.

Speir and Ross (1978) discussed soil sterilization in context of phosphatase activity in soils. They reported that the best method is to kill all microbial activity, thereby preventing assimilation of substrates and products and further synthesis of exoenzymes. They considered toluene and ionising radiation to be the effective treatments for sterilizing soil. The use of toluene as soil sterilant has however, been the source of some controversy. It can have effect on cell permeability (Jackson and De moss, 1965) and possibly soil structure (Cerna, 1972).

Since mineralisation of organic P and immobilization of inorganic P are of considerable importance for plant nutrition, they can be expected to affect the adsorption and desorption processes. This study was undertaken to evaluate the effect of various microbial inhibitors on soil P adsorption behaviour.

5.6.2 Materials and methods:

Three soils Dreghorn No.1 A, Caprington and Dunlop A were sterilized with following treatments. The procedures for soil sterilization with chloroform fumigation and autoclaving have been described in detail in Section 2.11.

1. Chloroform fumigation: Approximately 20 g of each soil was fumigated with purified chloroform in a large desiccator (Section 2.11.1).

2. Autoclaving: 20 g from each soil was autoclaved at a pressure 15 lbs /in^2 corresponding to 120^{o} C. Autoclaving was done for 30 minutes (Section 2.11.2).

3. Sodium azide 0.003M: The soils were not directly treated with sodium azide. 200 mg of sodium azide (NaN_3) was dissolved in 1 litre P treating solution.

4. τ-irradiation: The soil samples were irradiated with ^{60}Co source, for τ-irradiation, at the Scottish Universities Research and Reactor Centre.

5. Toluene: Two drops of toluene was added to the soil before treating with solution.

6. Control: Apart from above treatments, soil samples were run, without receiving any treatment, along with other samples.

For P adsorption studies 5 µg P cm^{-3} solution was tested with all treatments to investigate the effect of microbial inhibitors. Approximately 1.0000 g of soil from each treatment was accurately weighed into screw capped bottles. Each treatment was run in triplicate. 50 cm^3 of 5 µg P cm^{-3} solution was added to each bottle for all treatments except sodium azide treatment. For NaN_3 treatment, 50 cm^3 of treated P solution was added to each bottle. The bottles were screw capped and shaken for 24 h at 30°C on an orbital shaker and filtered through Whatman No 2 filter papers. The P concentration was determined colorimetrically using the Technicon Autoanalyzer II by the method described in Section 5.4.2, using a linear calibration curve with standards 0 and 5 µg P cm^{-3}.

P extraction was carried out with 0.5M acetic acid to evaluate the effect of microbial inhibitors on extractable P. Approximately 2.500 g of soil from each treatment was accurately weighed into screw capped bottles. Each treatment was run in triplicate. 50 cm^3 0.5M acetic acid was added to each bottle of all treatments except sodium azide treatment. For sodium azide treatment, 50 cm^3 of 0.5M acetic acid treated solution was added to each bottle. The procedure adopted was that described in Section 2.7.1.

The data collected in above experiments was analysed statistically. The comparison of means was carried out by the methods given by Gomez and Gomez (1984)

5.6.3 Results and discussion:

This study was undertaken to select an appropriate soil sterilant for preventing uptake or release of P by microbial biomass during adsorption. The relative effects of various soil sterilants on P adsorption were compared. The data on equilibrium P concentration was summarized in Table 5.9.

Table 5.9 Effect of soil sterilants on equilibrium P concentration in soil solution.

Treatments	Equilibrium P concentration (μg P cm^{-3})		
	Dreghorn No.1 A	Caprington A	Dunlop A
Control	2.71	3.67	0.89
Chloroform	3.04^{**}	3.80^{ns}	1.22^{**}
Na-azide	2.56^{*}	3.57^{ns}	0.70^{ns}
Toluene	2.82^{ns}	3.62^{ns}	0.89^{ns}
Autoclaving	3.09^{**}	3.99^{**}	1.37^{**}
τ-irradiation	2.87^{*}	3.73^{ns}	1.36^{**}

ns = Mean not significantly differs from control at 0.05

* = Mean significantly differs from control ($P < 0.05$)

** = Mean significantly differs from control ($P < 0.01$)

The result showed that autoclaving has significantly increased P concentration in all the soils. Chloroform also showed similar effect except in Caprington soil. This variable effects may be due to different P adsorption capacities of soils. Hedley and Stewart (1982) suggested that over a range of soils, recovery of microbial-P varies inversely with P adsorption capacity. The P concentration also increased significantly in Dreghorn No.1 A and Dunlop A soils with τ-irradiation, while this increase was not significant in Caprington soil.

It was observed in the present study that P concentration was decreased in all soils with sodium azide treatment. However, the decrease was significant in Dreghorn No.1 A soil and non-significant in other two soils. This may be due to rise in soil pH by sodium azide treatment as indicated by various workers (Rozycki and Bartha, 1981 and Wolf et al., 1989). Toluene had no effect in any of the soils.

From the analysis of results in P adsorption studies, an appropriate soil sterilant could not be selected, as there could be release of P from other sources due to various treatments. An extraction of P from the same treated soils was carried out with 0.5M acetic acid. The extractable P from various treatments was compared in Table 5.10. It was indicated from the results that the chloroform, and τ-irradiation had only

Table 5.10 Effect of soil sterilants on 0.5M acetic acid extractable P in soils.

Treatments	Acetic acid extractable P (μg P cm^{-3})		
	Dreghorn No.1 A	Caprington A	Dunlop A
Control	7.6	28.4	10.2
Chloroform	8.0^{ns}	28.6^{ns}	12.5^{**}
Na-azide	7.2^{ns}	28.0^{ns}	9.2^{ns}
Toluene	8.0^{ns}	30.7^{ns}	11.1^{ns}
Autoclaving	8.7^{ns}	38.1^{**}	11.5^{*}
τ-irradiation	8.0^{ns}	29.2^{ns}	12.7^{**}

ns = Mean not significantly differs from control at 0.05

* = Mean significantly differs from control ($P < 0.05$)

** = Mean significantly differs from control ($P < 0.01$)

significant increase in extractable P in Dunlop A soil, while autoclaving significantly increased extractable P in Dunlop A as well as in Caprington A soil over control. No significant difference was observed due to toluene and NaN_3 treatments against control.

Autoclaving, τ-irradiation and chloroform in general, increased the extractable P over control in all the soils indicating that P from some other sources released. Brookes et al., (1982) pointed out that in addition to microbial biomass P, 40% of the P is rendered extractable by $CHCl_3$. Jakobsen and Andersen (1982) also reported an elevated concentration of P and N after soil irradiation. While Cline et al., (1985) found no effect of irradiation on water extractable-P. Though they found much greater effect of autoclaving on indigenous soil P than irradiation, yet no consistent differences were observed between sterile and non-sterile soils. However, in the present study, the aim was just to kill the microorganisms responsible for utilizing P which otherwise may be adsorbed or desorbed. Olsen and Watanabe (1957) reported that growth of microorganisms interfered with inorganic phosphorus reactions for the 3-day period. White (1980) reported that the mechanism of P adsorption is affected by microbial uptake of P, which becomes significant within 2-4 hours at 25^{o} C.

It can be concluded from the studies that toluene and NaN_3 could be the soil sterilants for P adsorption

studies. The former could be preferred as it can be used conveniently and some problems associated with the use of NaN_3 have also been pointed out by Rozycki and Bartha (1981). 2-3 drops of toluene for each soil sample have been used by many workers to stop microbial activity in P adsorption and desorption studies (Olsen and Watanabe, 1957; Rajan, 1973; Oskay, 1986; Gama et al., 1987 and Loganathan et al., 1987)

The effect of toluene was tested in a P adsorption experiment on three soils. In this study the soils were equilibrated with 5 µg P cm^{-3} for 72 hours. Final equilibrium P concentration is presented in Table 5.11. The results showed that the toluene has only affected significantly P adsorption in Dunlop A soil, while its effect in other two soils was non-significant.

Table 5.11 The effect of toluene as soil sterilant on soil P adsorption

SOILS	Equilibrium P concentration (µg P cm^{-3})		
	Toluene	No toluene	T-test
DREGHORN No.1 A	2.78	2.83	Ns
CAPRINGTON A	3.74	3.57	NS
DUNLOP A	1.09	0.97	*

* = Significantly differ at 0.05
NS = Non significantly differ at 0.05

CHAPTER 6

Availability of phosphate to ryegrass

6.1.1 Introduction

The limitations in the availability of phosphorus to plants arise from various processes of interaction of this nutrient element with both soil constituents and plants. The interaction in the soil system is of more significance since it determines, in the first instance, how much phosphorus is accessible to plants. Soil minerals can immobilize most of the added phosphorus, thereby reducing its availability to growing plants. The degree of phosphorus immobilization, and therefore the degree of availability of applied phosphorus to plants, varies from soil to soil and depends on different factors like initial phosphorus status, soil texture, organic matter content, the nature of soil minerals, interacting ions and the affinity of phosphate for the various adsorbing surfaces.

Numerous studies have shown that phosphate behaviour is dominated by strong specific adsorption in soils. This results in a very low concentration in soil solution. The very low concentration of orthophosphate (often less than 1 μg P cm^{-3}) maintained in the soil solution (Archer, 1988) coupled with the considerable demand from plants

necessitates the determination of P availability to plants.

The most important mechanism involved in the movement of phosphate to absorbing roots is diffusion. Many researchers agreed that phosphate moves to the roots of plants by diffusion (Barber, 1962; Olsen and Watanabe, 1963; Lewis and Quirk, 1967). Thus the concentration of phosphate in the soil solution is vital, since the soil solution must contain sufficient phosphorus to provide the concentration gradient necessary for net movement to the root.

Maintenance of suitable concentration of phosphorus in the soil solution (intensity) depends on solid phase phosphorus going into the solution to replace the amounts withdrawn by plant uptake. The quantity of solid phase phosphorus is frequently referred to as the capacity or quantity factor. Both intensity and quantity parameters determine the soil buffering capacity (Barrow, 1967; Holford, 1976). Soil P movement can be presented by following relationship:

Soil solution P<===>Labile soil P<===> Nonlabile P

The soil phosphorus quantity factor includes both labile and nonlabile forms of phosphorus. Labile soil P is the readily available portion of the quantity factor. Depletion of labile P usually causes nonlabile P to become labile, but at a very slow rate (Tisdale et al., 1985).

Whatever the mechanism by which plants obtain phosphates from the soil, the important factor to be considered is the supply of phosphorus above a critical level. The required concentration of phosphorus in soil solution necessary for plant growth depends primarily on the crop species being grown. Many research workers have indicated that concentration of 0.2 to 0.3 μg P cm^{-3} in soil solution are adequate for a variety of crops (Beckwith, 1964; Ozanne and Shaw, 1968; Fox and Kamprath, 1970). However, Fox et al. (1986) suggested 0.1 and 0.2 μg P cm^{-3} in the soil solution for ryegrass and white clover respectively..

Mays et al. (1980) reported that in most situations P concentration in grasses below about 0.2% indicates a deficiency for plant growth, while 0.3 to 0.35% is usually necessary for optimum yields. Archer (1988) reported that when soil phosphorus level is satisfactory, the leaf levels of phosphorus in plants are commonly 0.3 to 0.4%, while less than half of this value may give rise to severe deficiency.

In the following study, the influence of P uptake by ryegrass on phosphate adsorption and extractable P in a pot experiment under controlled environmental conditions has been examined. In addition to the P status of soils, enzymes activities were also measured before and after the experiment.

6.1.2 Materials and methods

6.1.2.1 Pot experiment technique

To study the effect of P uptake by plants on the phosphorus status of soils, a greenhouse experiment was carried out. Five representative soils from the group of soils already studied for P adsorption and P extraction were re-sampled. These soils were Dreghorn No.1 A, Darvel A, Caprington A, Dunlop A and Midelney A (Grass). The sampling sites have already been described in Chapter 2. After sieving through 4 mm sieve, approximately 1 kg of each fresh soil sample was kept in the cold room at 2^{o}C for analysis. The remaining fresh soil samples were used in the pots. The soil moisture content of fresh soils was determined (Section 2.2). The enzymic activities were assessed in samples of the fresh soil (Section 2.10). The soils were air dried for determination of extractable P and P adsorption isotherms according to the methods described in Section 2.7 and Section 2.8.2.1 respectively. Each plastic pot (4 inch diameter) was filled with fresh soil to the top. The experiment was laid out in a Latin Square design with two main blocks (Fig 6.1). In one block grass was sown while the other block had no grass. Each soil was replicated randomly five times in a block. As the grass was sown in the short daylight season of winter, supplementary lighting and heating were arranged. To enhance the photosynthetic activity artificial light (fluorescent tubes) was set on

the pots for 16 hours per day to supplement the natural light. Temperature was maintained at between 10-20^{o} C.

Ryegrass (Lolium perenne) seed was sown at the rate of 0.5 g per pot. After sowing the pots were covered with a black polyethylene sheet to enhance germination. After complete germination the pots were placed in saucers on a bench according to the layout given in Fig 6.1. Deionized water was applied regularly to each pot from the saucers according to requirement of each soil. Some weeds which appeared in the non-grassed pots were removed.

Nitrogen, potassium and magnesium were applied to each pot one week after emergence of the seedlings at the rate of 100, 50, and 5 mg per pot respectively, as prescribed by Bakhsh (1988). The source of nutrients were 28.6g of NH_4NO_3 for N, 19.1g of KCl for K and 5.06g of $MgSO_4.7H_2O$ for Mg. All the above nutrients were dissolved in deionized water, the volume was made to 1 litre. From this nutrient solution, 10 cm^3 was applied to each pot with 10 cm^3 pipette uniformly. All the non-grass pots were treated in the same way as the grassed pots.

12 weeks after sowing the watering was stopped, the grass was cut just above the soil surface. The fresh yield of tops (leaves + stubbles) was obtained by weighing. Roots were separated from soils by washing thoroughly with deionized water to remove adhering soil materials.

Figure 6.1 LAYOUT: LATIN SQUARE DESIGN

Block-1 GRASSED

D4	C3	A1	E5	B2
E2	D1	B4	A3	C5
A5	E4	C2	B1	D3
C1	B5	E3	D2	A4
B3	A2	D5	C4	E1

Block-2 NON-GRASSED

C5	E2	D1	B4	A3
D3	A5	E4	C2	B1
B2	D4	C3	A1	E5
A4	C1	B5	E3	D2
E1	B3	A2	D5	C4

A = DREGHORN NO.1 A B = CAPRINGTON A C = DUNLOP A

D = DARVEL A E = MIDELNEY (GRASS)

Total tops and roots were dried separately in an oven at 85^{o} C for 16 hours (Jones, 1990) for measurement of dry matter yield. Roots and tops were ground separately with an electrical grinder and stored in sealed plastic bags for further chemical analysis.

Soil from each pot was collected in polyethylene bags and stored in a cold room for further chemical analysis.

6.1.2.2 Analytical methods

Soils were characterized before the grass was grown and after the grass was harvested. pH and soil moisture contents were determined by the methods given in Section 2.1. and 2.2. respectively. Phosphorus was extracted with 0.5 M acetic acid, 0.5 M $NaHCO_3$, and acidified NH_4F by the procedures already described in Section 2.7. The adsorption isotherms of all soils were determined before and after the experiment, by the method given in Section 2.8.2.1 The P concentration in solutions obtained in the extraction and adsorption studies, was determined colorimetrically by Technicon Autoanalyzer II (Section 2.6.2). Acid and alkaline phosphatase and phosphodiesterase activities were also assessed by the procedures in Section 2.10. For the analysis of plant material, the Kjeldahl method of digestion was used (Section 2.9.). The plant digest was analysed for P by Technicon Autoanalyzer II, using ammonium metavanado-

molybdate method (Section 2.6.3). The experimental results were statistically analysed and separation of means was done by the methods given by Gomez and Gomez (1984).

6.1.3 Results and discussion

6.1.3.1 Crop yield and phosphorus uptake:

Dry matter yield and P uptake were used to evaluate the availability of residual soil P. It is recognized that there should be differences in dry matter production due to inherent differences between soils. However, comparison of P removal, extractable P and P adsorption behaviour were studied as means to evaluate the availability of P in a group of soils.

The results showed that there were significant differences in dry matter yield of tops and roots of plants (Table 6.1). The greatest yields of tops were in Darvel A and Midelney (Grass) A soils, followed by Caprington A soil. The Dreghorn No.1 A and Dunlop A soils produced the lowest dry matter yield of tops. The dry matter yield of roots was significantly highest in Darvel soil.

Total P uptake was measured in leaves as well as in roots (Table 6.1). The analyses of data showed significant differences in P uptake from different soils. The grass grown on the Midelney (Grass) soil

Table 6.1 Average yield of ryegrass obtained in pot experiment and P uptake

Soils	Dry matter yield in g/pot			P uptake in %	
	Tops	Roots	Total	Leaves	Roots
DREGHORN No.1 A	1.84 c	0.804 bc	2.64 d	0.140 c	0.162 bc
CAPRINGTON A	3.01 b	0.874 bc	3.89 c	0.188 b	0.185 ab
DUNLOP A	1.32 c	0.626 c	1.95 d	0.122 c	0.116 d
DARVEL A	4.36 a	1.804 a	6.16 a	0.216 b	0.160 c
MIDELNEY (GRASS)	4.07 a	1.144 b	5.22 b	0.276 a	0.209 a

Means in a column followed by similar letter(s) did not differ significantly at 5% level of probability.

had highest concentration of P in leaves as well as in roots, while that on the Dunlop soil had the lowest. The uptake of P has shown a profound effect on the crop yield (Fig 6.2). It was observed that the dry matter yield of leaves increased with increasing P concentration in leaves. Mattingly et al. (1963) reported an increase of dry matter yield in ryegrass with increase in P uptake. A highly significant correlation between P uptake and dry matter yield of maize ($r = 0.879^{**}$) was observed by Patiram and Prasad (1990).

The extractable P lost from the soils during grass growing period and the P removed by the plants are summarized in Table 6.2. The results showed that the P removed by the grass was not merely the extractable P, but it also included P which was made available to plants from some non-labile sources. Kamprath and Watson (1980) reported significant relationship of concentration of P in plant tissues and soil P removed by various extractants.

Table 6.2 Mean extractable P decreased from soils and P removed by plants

SOILS	Extractable P decreased from soils (mg/pot)			P in plant (mg/pot)
	Acetic acid	Bray-I	Sodium bi-carbonate	
Dreghorn	0.46	-0.36	0.29	3.84
Caprington	0.84	0.79	1.13	7.23
Dunlop	0.07	0.18	0.12	2.32
Darvel	1.23	3.49	3.08	12.30
Midelney (Grass)	7.15	8.48	3.76	13.60

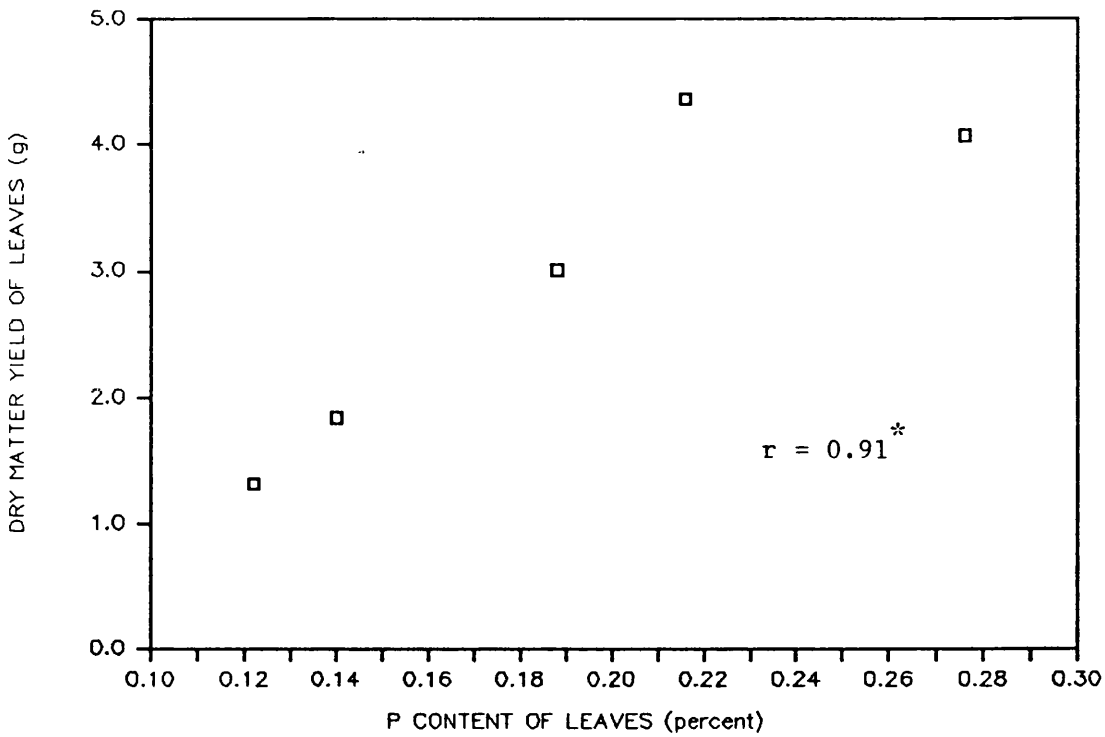


Fig. 6.2 Effect of P concentration in leaves on dry matter yield

6.1.3.2 Relationship of plant response with some soil characteristics before planting

The coefficients of correlation for pH, various soil-P test methods and enzymic activities in soils before planting with P-uptake and dry matter (Top) yield are presented in Table 6.3. It was revealed from the results that sodium bicarbonate extractable P has significant correlation with P uptake and dry matter in leaves of ryegrass (Fig. 6.3 and Fig. 6.4). The other two test methods showed non-significant correlation with P uptake and dry matter yield. The correlation studies in this case showed that sodium bicarbonate extractable P was the best predictor of dry matter yield and P content of the ryegrass. Some other workers have also reported that Olsen's sodium bicarbonate method is better in estimating the plant availability of phosphorus.

Mattingly et al (1963) regarded the 0.5 M sodium bicarbonate as the best laboratory test, which gave highest correlation ($r = 0.920$) between soil P and P uptake by ryegrass in a group of soils. Of all the methods tested Olsen's proved least sensitive to changes in soil properties, giving reliable estimates of soil phosphorus availability in calcareous soils as well as in acid soils (Walmsley and Cornforth, 1973). Part of its success may be due to its ability to dissolve some of the aluminium and iron bound phosphate as well as phosphate associated with hydrous oxides (Williams and Knight, 1963;

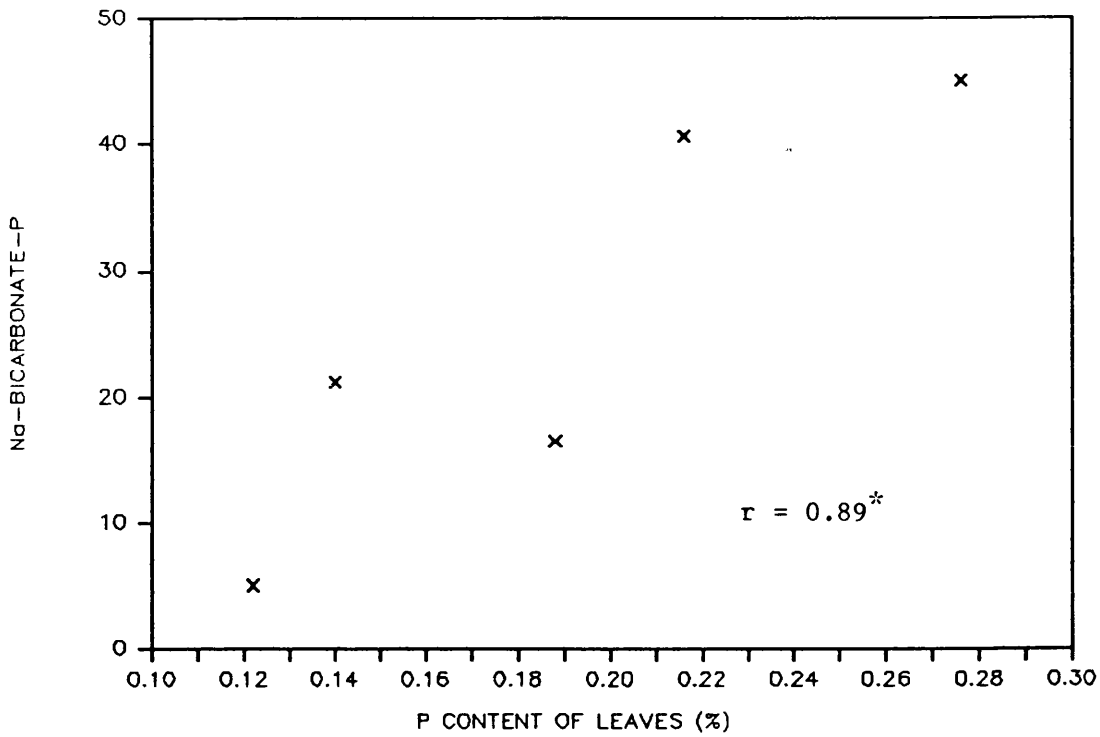


Fig. 6.3 Scatter diagram showing the relationship between sodium bicarbonate extractable P and concentration of P in leaves.

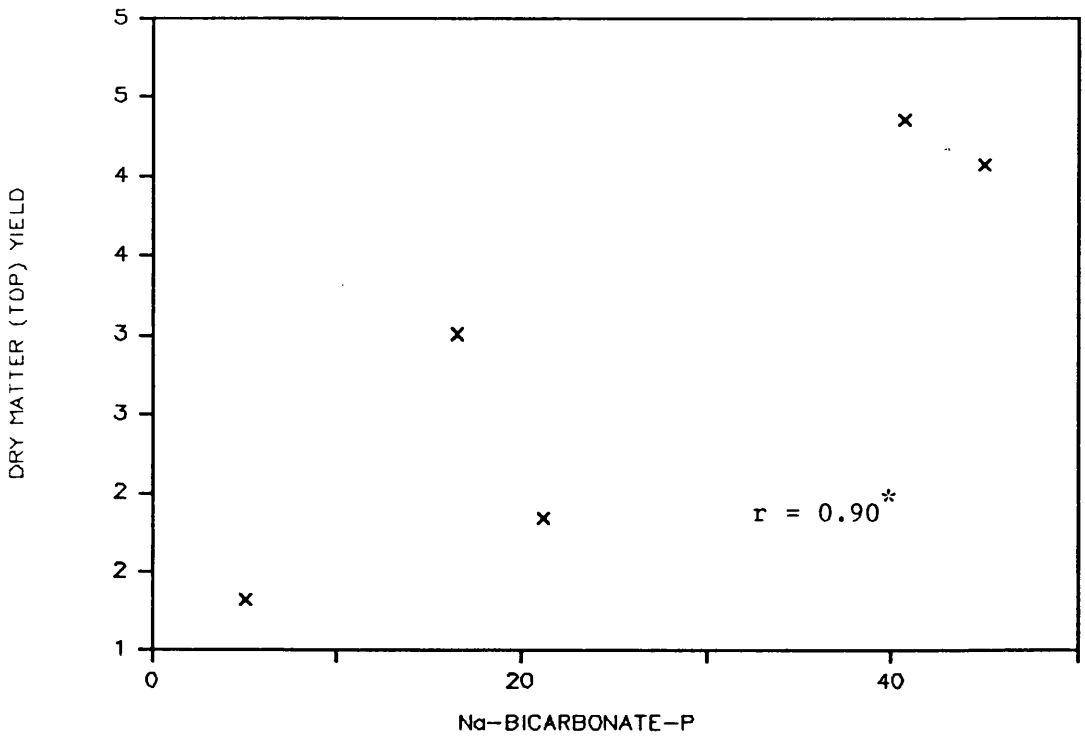


Fig. 6.4 Scatter diagram showing the relationship between sodium bicarbonate extractable P and dry matter yield of leaves.

Table 6.3 Linear correlation of some soil characteristics before planting, dry matter yield and P uptake by the ryegrass in 5 soils.

	Dry matter (Top) yield	P uptake
pH	0.31^{NS}	0.60^{NS}
Acetic acid extractable P	0.53^{NS}	0.83^{NS}
$NaHCO_3$ extractable P	0.90^{*}	0.89^{*}
Bray-I extractable P	0.81^{NS}	0.72^{NS}
Acid phosphatase	-0.5^{NS}	-0.45^{NS}
Alkaline phosphatase	0.49^{NS}	0.78^{NS}
Phosphodiesterase	0.49^{NS}	0.79^{NS}
P uptake	0.91^{*}	-

NS = Not significant at 5% level of probability
* = Significant at 5% level of probability

Horneck et al., 1990). Several other workers have recommended Olsen's method for tropical soils, as giving the best indication of crop yield response and P uptake both in greenhouse and field experiments (Gupta and Cornfield, 1963; Mehta and Patel,1963; Jackson et al., 1964). Ekpete (1976) found the Olsen's method the most effective in extracting phosphorus from both water-logged and air dry soils and was highly correlated with yield response of rice. Hence it was suggested that the Olsen's method represented a relatively accurate index of the phosphorus available to rice. Holford (1980) showed that the Olsen's test exhibits a similar sensitivity to buffering as plants, thus its superiority over a range of soils. The superiority of Olsen method over Bray method in terms of better correlation with P uptake has been reported in the literature (Holford, 1980; Sims and Ellis, 1983; Yerokun, 1987). Olsen's sodium bicarbonate method has been considered the best method in Britain on which to base advice to farmers on fertilizer use (wild, 1988). Sharpley (1991) concluded that both resin and bicarbonate extractable P represent plant available P forms, probably physically sorbed by the soil material. As a measure of P availability, based on correlation of extractable P with P uptake and yield increase, $NaHCO_3$ extractant was effective and superior to other acidic extractants including Bray-I method (Bates, 1990).

In the present studies the other two tests showed poor correlation with dry matter yield as well as with P

uptake (Table 6.3). Poor correlation between yield of ryegrass and acetic acid extractable P was also reported by other research workers (Mattingly and Pinkerton, 1961; Mattingly et al., 1963; Williams and Knight, 1963). Mattingly et al. (1963) suggested that the poor correlation between acetic acid extractable P and crop P uptake is due to the fact that acetic acid attacks phosphorus sources not available to plants. An overestimation of phosphate extracted by Bray-I method has been reported by some workers (Chien, 1978; Yerokun, 1987 and Menon et al., 1990. Though the active fluoride ion coupled with strong acid (HCl) in Bray-I enables it to attack all forms of phosphates not available to plants (Williams and Knight, 1963), this method is regarded most successful in areas of wide range of acid soils (Olsen and Sommers, 1982; Patiram and Prasad, 1990). Highly significant correlation ($r=0.8^{**}$) between Olsen P and Bray-I P in some soils has been reported by Horneck et al. (1990).

6.1.3.3 Effect of plant growth on phosphate extracted from soils by three extractants.

At the start of pot experiment before planting the soils were characterized for extractable P by three commonly used extractants. After harvesting, P was extracted by the same three methods. The results for each extractant are given in Table 6.4. From the statistical analysis it was observed that the acetic acid extractable

Table 6.4 PO_4-P extracted from soils by three extractants before and after planting

SOILS	Extractable-P in μg g^{-1} soil		
	Before planting	After harvesting	
		Grassed pots	Non-grassed pots
0.5M Acetic acid extractable P			
DREGHORN No.1 A	5.8 a	4.7 b	5.9 a
CAPRINGTON A	12.6 a	10.5 b	12.9 a
DUNLOP A	1.7 ab	1.5 b	1.8 a
DARVEL A	9.0 a	5.9 c	8.3 b
MIDELNEY (GRASS)	107.8 a	84.0 b	104.5 a
0.5M $NaHCO_3$ extractable P			
DREGHORN No.1 A	21.2 b	20.5 b	23.0 a
CAPRINGTON A	16.5 b	13.7 c	19.1 a
DUNLOP A	5.1 a	4.7 a	4.9 a
DARVEL A	40.7 a	33.0 c	39.2 b
MIDELNEY (GRASS)	45.0 a	32.4 b	46.3 a
Bray-I extractable P			
DREGHORN No.1 A	57.0 b	57.9 b	64.1 a
CAPRINGTON A	26.7 b	24.7 b	31.7 a
DUNLOP A	23.7 b	23.1 b	25.2 a
DARVEL A	107.6 b	98.9 c	119.5 a
MIDELNEY (GRASS)	92.6 a	64.3 b	91.1 a

Means in a row followed by similar letter(s) did not differ significantly at 5% level of probability

P in non-grassed controls was comparable in all soils except Darvel. The acetic acid extractable P in grassed pots was lower. It suggests that the 0.5M acetic acid is a good measure of P utilized by the grass.

In case of Bray-I extractant, higher P in all non-grassed soils than original soils suggests changes in this fraction of P. Bray-I extractable P in the grassed soils was same as or less than the P extracted from the soils before planting. It may be result of competitive processes reflected by the extractant.

Sodium bicarbonate extractable P was lower in grassed soils than all non-grassed soils except Dunlop. There was variable effect in the extractable P of soils before planting.

6.1.3.4 Effect of plant growth on soil phosphate adsorption

The phosphate extracted by chemical extractants must be affected both by the amount and by the nature of the phosphate present in the soil and it must be affected by the capacity of soil to adsorb phosphate from solution. Strong retention of phosphate by the soils depends upon the amount of active compounds of iron, aluminium and calcium and on organic matter content in the soils. In the following investigations phosphate adsorption isotherms for soils used in the pot experiment were measured (Figs. 6.5 to 6.9).

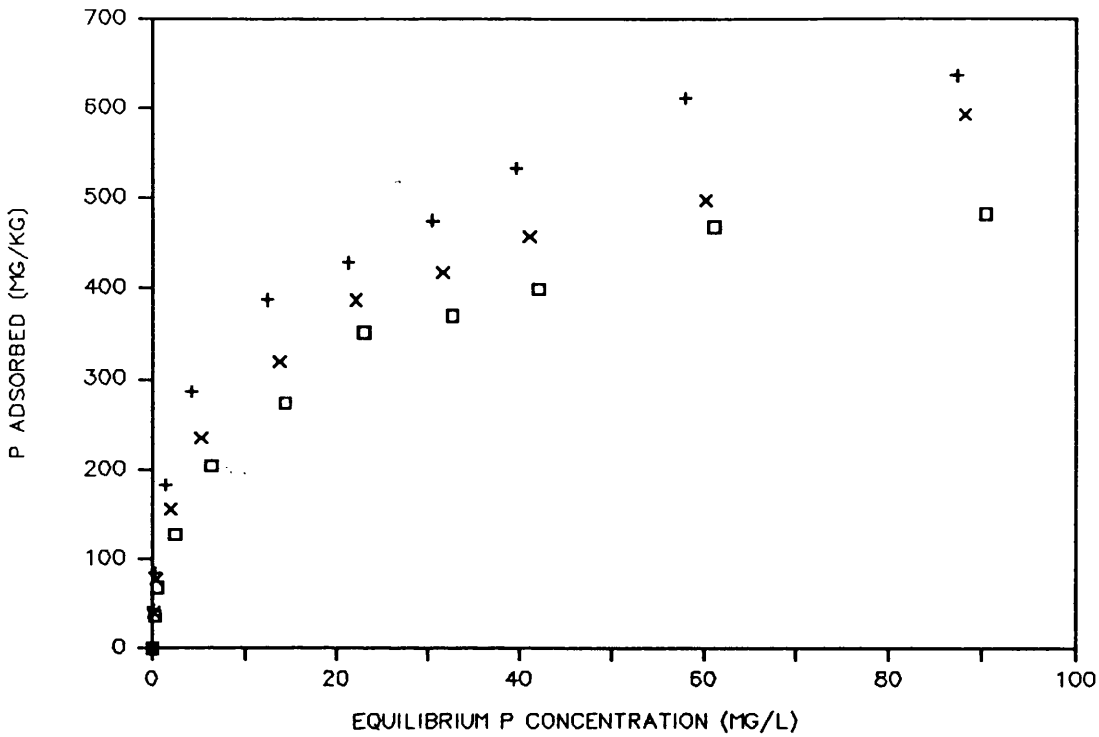


Fig. 6.5 Effect of plant growth on P adsorption isotherms for Dreghorn No 1 A soil.

(x) = Grassed pots

(+) = Non-Grassed pots

(□) = Original soil

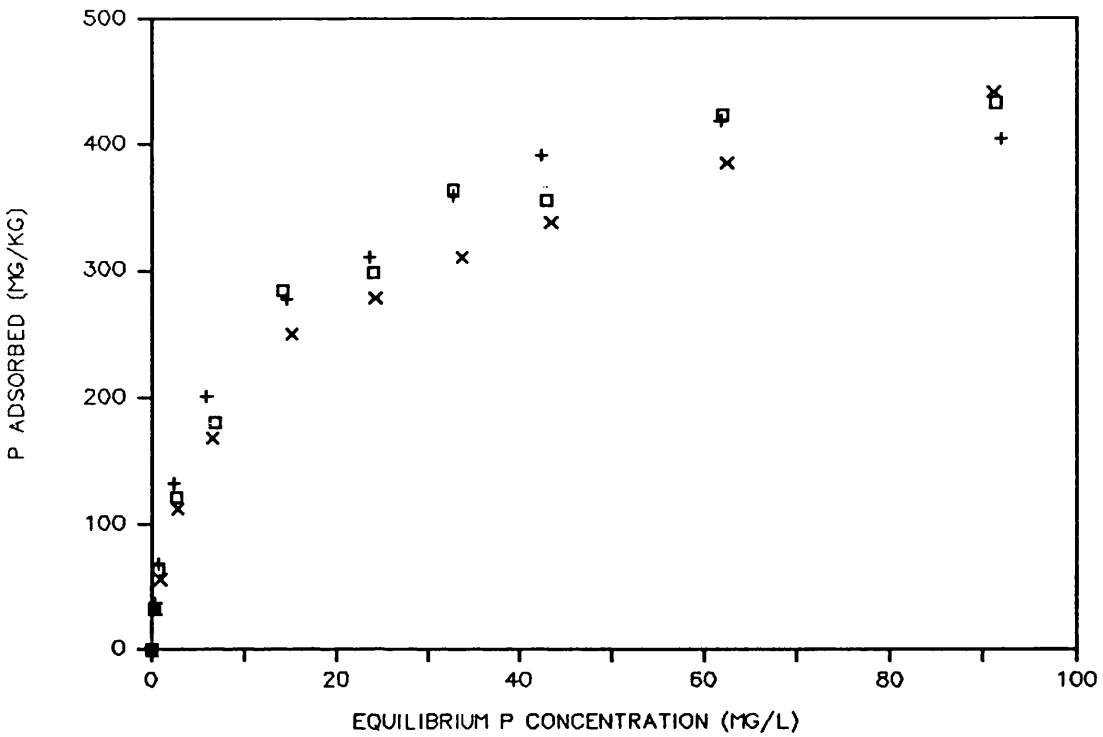


Fig. 6.6 Effect of plant growth on P adsorption isotherms for Caprington A soil.

(x) = Grassed pots

(+) = Non-Grassed pots

(□) = Original soil

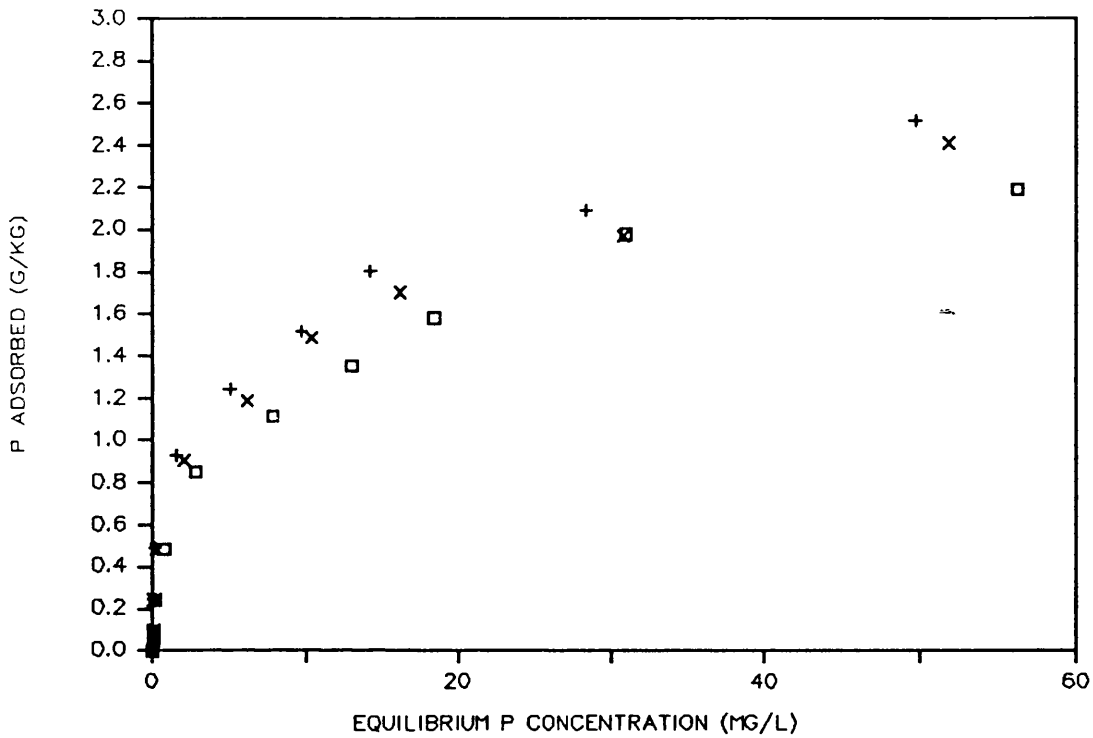


Fig. 6.7 Effect of plant growth on P adsorption isotherms for Dunlop A soil.

(x) = Grassed pots

(+) = Non-Grassed pots

(□) = Original soil

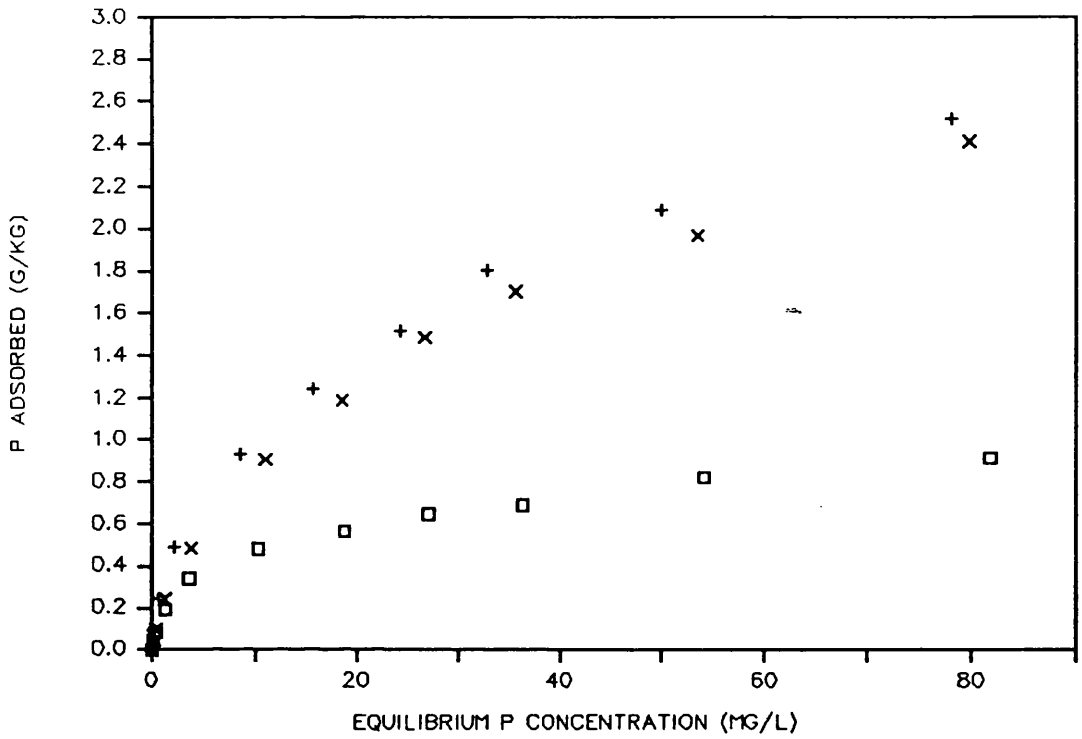


Fig. 6.8 Effect of plant growth on P adsorption isotherms for Darvel A soil.

(x) = Grassed pots

(+) = Non-Grassed pots

(□) = Original soil

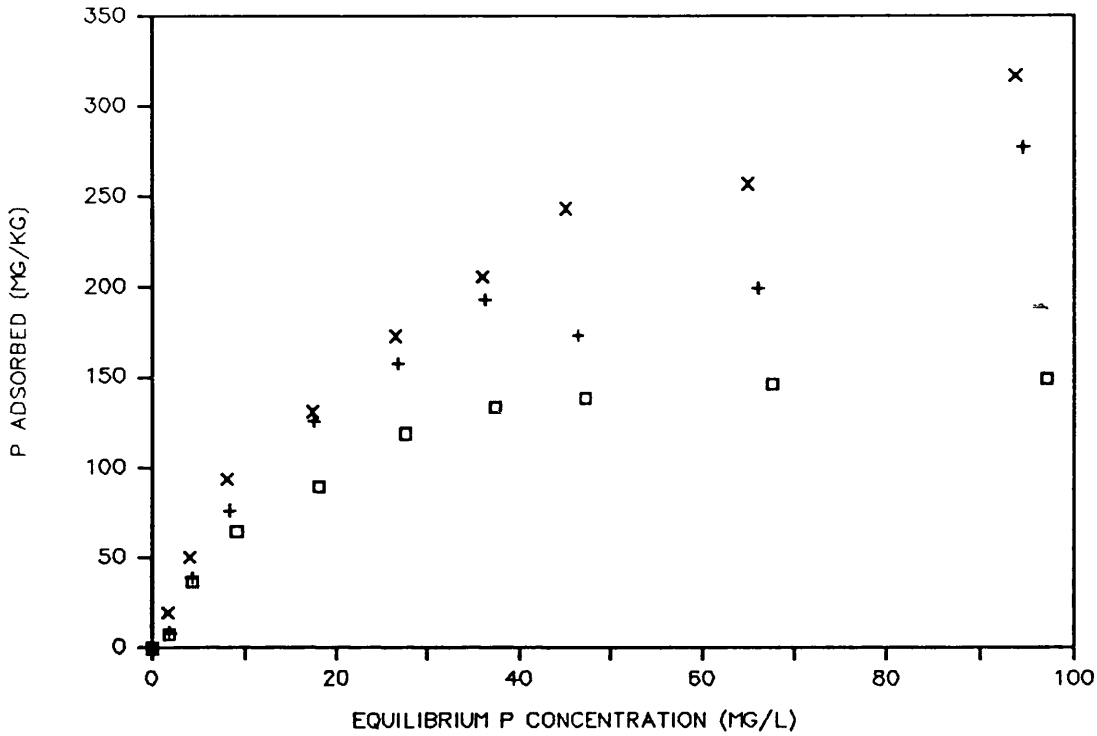


Fig. 6.9 Effect of plant growth on P adsorption isotherms for Midelney (Grass) soil.

(x) = Grassed pots

(+) = Non-Grassed pots

(□) = Original soil

The Langmuir and Freundlich equations were applied to the data and the constants were calculated according to the formulae given in Chapter 4. The values of constants and R^2 are given in Table 6.5.

The adsorption maxima were compared in a regression analysis, it was observed that there was no significant difference in the adsorption maxima of soils before and after planting except Dreghorn and Midelney (grass).

Table 6.5 Langmuir and Freundlich constants in pot experiment

	Langmuir plots			Freundlich plots		
	Adsorption maximum (µg/g)	Bonding energy ($1\ mg^{-1}$)	R^2 %	Slope	Intercept	R^2 %
DREGHORN No.1 A						
BEF	513b	0.122	98.6	0.434	0.61	98.5
NG	653a	0.172	98.3	0.386	0.97	97.3
G	591ab	0.128	97.3	0.406	0.80	98.5
CAPRINGTON A						
BEF	461a	0.123	98.8	0.449	0.50	97.2
NG	433a	0.180	99.4	0.410	0.68	97.2
G	459a	0.094	97.6	0.459	0.42	98.6
DUNLOP A						
BEF	2257a	0.228	97.7	0.506	1.06	94.5
NG	2512a	0.326	97.9	0.474	1.29	88.0
G	2392a	0.298	97.5	0.472	1.26	91.2
DARVEL A						
BEF	934a	0.136	97.8	0.466	0.75	96.7
NG	1111a	0.191	98.3	0.436	0.99	95.5
G	1030a	0.104	96.2	0.494	0.63	97.3
MIDELNEY (GRASS)						
BEF	196b	0.042	92.4	0.692	-1.09	86.8
NG	400a	0.020	77.2	0.789	-1.38	90.6
G	408a	0.031	98.0	0.921	-1.89	86.3

The adsorption maximum values followed by similar letter(s) did not significantly differ at 0.05 level of probability

BEF = Before planting soils; NG = Ungrassed soil;
G = Grassed soil

6.2 Effect of plant growth on phosphatase enzyme activities in soil

6.2.1 Introduction

Phosphatase enzymes play a major role in the mineralisation of organic phosphates in soil. Soil is a dynamic living system where all biochemical activities proceed through enzymatic process (Tabatabai, 1982). Enzymes accumulated in soils are present as free enzymes, such as exoenzymes released from living cells, endoenzymes released from disintegrating cells and enzymes bound to disintegrating cell constituents (Kiss et al., 1975).

The transformation of soil phosphorus is not well understood but it is generally accepted that phosphorus is taken up by the plant roots as inorganic phosphate (Somani, 1989; Herbien and Neal, 1990b). Since a large proportion of the total soil phosphorus (30-70 %) is bound in complex organic molecules (Skujins, 1978), the mineralisation of the organic P fraction can be an important factor in plant nutrition. Mineralisation appears to be catalysed by root exocellular enzymes which may be either liberated into the soil solution or bound to the root epidermal cells but outside their permeability barrier (Speir and Ross, 1978).

Addition of an energy source containing readily assimilable carbon increased the rate of mineralisation (Halm et al., 1972), indicating that microbial

proliferation is at least partly responsible. However, the mineralisation of soil organic phosphorus is associated with the mineralisation of organic matter as whole. Speir and Ross (1978) reported that if the phosphorus concentration in organic matter being assimilated by microorganisms is below 0.2 to 0.3 percent, then no phosphorus will be mineralized in microbial tissue.

It is still uncertain to what degree the various phosphorus compounds can be utilized by plants, particularly the organic P fraction in soil. However the labile organic phosphorus compounds are mineralized in soils by enzymes, collectively called phosphatases, that catalyse the hydrolysis of esters and anhydrides of phosphoric acid (Speir and Ross, 1978). Sharpley (1985) has the view that plants can utilize organic P fractions from the soil by means of phosphatase activity enriched in the soil-root interface. Among the soil phosphatases, phosphomonoesterases and to lesser extent phosphodiesterases, have been the most widely studied. The phosphomonoesterases are further classified into acid and alkaline phosphatases, because they show their optimum activities in acid and alkaline ranges respectively. Both acid and alkaline phosphomonoesterases may exist in soils (Mathur and Rayment, 1977; Juma and Tabatabai, 1978) or only one or the other (Eivazi and Tabatabai, 1977; Herbien and Neal, 1990a).

Phosphodiesterase activity in soil is usually reported optimum at the pH ranging from 9 to 11 (Eivazi and Tabatabai, 1977).

The objective of this study was: (1) to assay the enzymes activities in the soils before planting and after harvesting in both the grassed and ungrassed soils (2) to find the effect of plant growth on enzyme activities.

6.2.2 Results and discussion

6.2.2.1 Phosphatases activities in pot experiment:

The activities of all the three phosphatases were measured before planting and after harvesting in grassed and in non-grassed pots. The results are given in Table 6.6. The data showed significant differences in the enzymes activities in all the soils except in Dreghorn soil for alkaline phosphatase and phosphodiesterase. The enzyme activities generally increased in grassed soils as compared to non-grassed pot soils with a variable differences to original soils. However, the results revealed an effect of plant growth on enzyme activities. Specific stimulation of phosphatase-producing microorganisms by the roots had early been reported by Greaves and Webley (1965). Plant roots are known to contain phosphatases which have the ability to induce hydrolysis of certain organic phosphates (Gilliam, 1970; McLachlan, 1976). Tarafdar and Jungk (1987) reported that rhizosphere of all the crops tested had higher

Table 6.6 Enzymic activities and pH of soils used in pot experiment before planting and after harvesting.

	DRG	CAP	DUN	DAR	MID
(a) Enzymic activities (μ moles pnp g^{-1} soil per hour)					
(1) Acid phosphatase					
Before	2.42 b	3.84 b	4.84 a	2.84 ab	2.90 b
Non-grassed	2.94 a	3.42 b	4.07 b	2.68 b	3.56 a
Grassed	2.80 ab	3.96 a	5.31 a	3.05 a	3.60 a
(2) Alkaline phosphatase					
Before	0.30 a	2.15 a	1.31 b	0.94 a	4.15 b
Non-grassed	0.34 a	1.49 b	1.33 b	0.78 ab	3.85 b
Grassed	0.21 a	1.97 a	1.60 a	0.76 b	4.70 a
(3) Phosphodiesterase					
Before	0.11 a	0.75 a	0.29 b	0.22 b	1.35 a
Non-grassed	0.22 a	0.34 b	0.23 c	0.16 c	0.72 c
Grassed	0.18 a	0.83 a	0.39 a	0.25 a	1.14 b
(b) pH of soils					
Before	4.9	5.6	5.8	5.3	6.4
Non-grassed	4.1	4.8	4.4	4.7	5.6
Grassed	4.4	5.9	4.7	5.5	6.6

Means in a column of an enzyme followed by similar letter(s)
did not differ significantly at 0.05 level of probability

DRG = Dreghorn No 1 A; CAP = Caprington A; DUN = Dunlop A

DAR = Darvel A; MID = Midelney (Grass)

levels of both acid and alkaline phosphatase than the bulk soil, due to the increased microbial and root activity in this zone.

By comparing the activities of different enzymes it was shown that the activity of acid phosphomonoesterase was higher than the other two enzymes. It supports the findings of earlier workers who suggested that acid phosphatase is predominant in acid soils and alkaline phosphatase is predominant in alkaline soils (Eivazi and Tabatabai, 1977; Juma and Tabatabai, 1978 and Tabatabai, 1982;).

Phosphodiesterase activity was considerably less in all soils examined than both phosphomonoesterases. The results are consistent with the findings of Herbien and Neal (1990b), who reported that active hydrolysis of organic P to inorganic P, by phosphodiesterase would not be expected to occur at maximal rate in acid soils. Eivazi and Tabatabai (1977) and Browman and Tabatabai (1978) have also argued that phosphodiesterase is maximum in soils buffered at a pH of 9.0 to 11.0.

Different soils varied in their enzymic activities according to their nature of origin. From the perusal of data it has been observed that acid phosphatase activity in Dunlop A soil was the highest among these soils. The maximum value in this case may be attributed due to the highest organic matter content (Table 2.2). The results

are consistent with Herbien and Neal (1990a) who found phosphomonoesterase activity 10-times greater than phosphodiesterase in the same soil. They suggested that this may reflect the type and quantity of organic substrate available for mineralisation. The other two enzymes under study have shown their maximum activity in the Midelney (Grass) soil. It may be due to the finer texture of soil and maximum value of pH, which encouraged the alkaline buffered enzymes. Tarafdar and Jungk (1987) observed similar effects in alkaline phosphatase, while comparing different soils. Herbien and Neal (1990a) observed that soil pH also had an effect upon the distribution of enzymes activity. They found the activity of phosphomonoesterase at pH 6.5-7.0 was 2 times less than at pH 4.6, while phosphodiesterase activity in acidic soils was optimum near pH 6.

CHAPTER 7

GENERAL CONCLUSIONS

The studies carried out have been concerned with phosphate behaviour in soils with different physico-chemical characteristics. Various aspects of phosphorus dynamics in soils and its availability to plants were studied.

7.1 Extractable P from soils

Available soil phosphate determined by various extractants, has been widely used for two purposes: (1) To evaluate the phosphorus status of soils, and (2) To serve as a basis for making quantitative fertilizer recommendations across a wide spectrum of soils. The determination of available phosphorus in any group of soils by different extractants frequently results in conclusions that differ in respect to the phosphorus status of the soils concerned.

In the present study six conventional extractants were used for 14 soils of different physico-chemical characteristics. The amount of phosphate extracted by various extractants varied significantly. The mean amount of extracted P from 14 soils ranged from 0.5 to 211 µg P

per g of soil. The P extracted by 0.5M sodium bicarbonate, sodium acetate buffer and ammonium acetate buffer was statistically comparable. Many workers have studied the correlation between results for available phosphorus as determined by various methods. In the correlation study of 6 extractants for 14 soils, sodium acetate buffer performed well in showing a very highly significant correlation with ammonium acetate, acetic acid and sodium bicarbonate and significant correlation with anion exchange resin. Bray-I extractant showed non-significant correlation with other extractants. The soils were split into two groups on the basis of origin as well as on pH. Sodium bicarbonate performance was relatively better in both groups of soils than other extractants. However, Bray-I extractant improved relationship with other extractants in Scottish soils. The performance of Bray-I, acetic acid and anion exchange resin was very poor in the slightly alkaline English soils. The literature does not show, however, any precise explanation of the differences in degree of correlation between different pairs of methods from the point of view of soil chemistry.

Soil phosphate determined by an extractant includes all forms of inorganic phosphates, but mainly aluminium, iron and calcium phosphate. The total and relative amounts of phosphorus dissolved from the various forms of phosphates depend on the relative amounts of calcium,

aluminium and iron phosphate in a soil, and relative solubilities of these phosphates in the extractants.

From the present study it may be concluded that due to the interactions between soils and extractants, it is not possible to find a universal method for extraction of P in all soil types. For better understanding of available soil phosphorus and for better agricultural practice of P fertilization it seems necessary to consider the intensity, quantity and capacity parameters.

7.2 Phosphate adsorbed by soils

Phosphorus needs of soils are based on the adsorption by soils of phosphate that is added in solution. The availability of soil phosphate is determined by the soil buffering capacity from a P adsorption isotherm. In the case of P fertilizer requirements phosphorus adsorption isotherms have been successfully used to estimate P fertilizer requirements of crops and as the basis for transferring information about P fertilizer requirements from one location to another. This approach of studying reactions of P fertilizers is more closely related to plant needs than some classical methods. The adsorption isotherms have advantages over conventional soil extraction methods, because the isotherms take into account both intensity and capacity factors.

In the present investigation, P adsorption isotherms were constructed for 14 soils. A very distinctive behaviour in adsorption isotherms was observed. Maximum P adsorbed by the soils ranged from 140 to 1400 μg P g^{-1} soil. The variations may be due to the history of fertilization, the pH value, soil texture and organic matter content of these soils. To summarize the adsorption properties of soils by using few parameters, to understand the nature of adsorption process and to get a value for adsorption maximum the P adsorption data were fitted to the Langmuir and Freundlich equations.

Using the R^2 values as a test for goodness of fit some of the soils conformed well to the Langmuir equation while other showed slight deviation at the lower end. The bending of lines toward the x-axis was thought to be due to the presence of strong adsorbing sites. The upward bending of line in some soils was due to the pre-occupied adsorbing surfaces by native or residual phosphates. The two useful parameters of P adsorption maximum and bonding energy constant were obtained from applying the Langmuir equation to the data.

By applying the Freundlich equation to the same P adsorption data, it was observed that most of the soils were good fit in terms of R^2. However, in some soils the line became gently curved at the top, indicating no further P adsorption at that point. Another

characteristics of the Freundlich equation was that the points at the top were compressed. It was thought that the poor linearity has been disguised in this compression.

To improve the R^2 values for goodness of fit some of the points at lower end for the Langmuir equation and some of the points at the top for the Freundlich equation were excluded from calculations. It was observed that in most the soils the amended data conformed relatively well to both equations than the previous data. By this practice the limitations of the Langmuir equation at the low end and for the Freundlich equation at the top end were revealed.

The relationship of some selected properties of soils with the Langmuir adsorption maxima were also observed. The pH and oxalate extractable Fe and Al have shown significant correlation with P adsorption maxima of 14 soils. However, the LOI, clay, fine sand and Fe were significantly correlated with P adsorption maxima in the Scottish soils. None of the soil properties in the English soils exhibited significant correlations with P adsorption maxima.

In the present study the Freundlich equation was better fit to the P adsorption data in most of the soils than the Langmuir equation. The R^2 values calculated from

the Freundlich plots of 14 soils were ranged from 87.8 to 99.3% (Table 4.2).

7.3 P adsorption and desorption isotherms

From initial study on P adsorption and desorption of 14 soils, it was realized that some errors occurred in the experimental technique. For this purpose 3 soils with different physico-chemical characteristics from amongst 14 soils were chosen for detailed study.

By plotting P adsorption and desorption isotherms on one graph for each soil, it was observed that the points were scattered and not following a trend in adsorption as well as in desorption isotherm. A wide gap between data points further created confusion in drawing a line. Some negative values on the desorption isotherms of two soils indicated some more P came into the system than the P actually added. Keeping in view the poor shapes of adsorption and desorption isotherms the experiment was redesigned.

Possible sources of errors in the experiment were assessed theoretically. The errors identified were then measured experimentally. The results were calculated in terms of standard deviation and confidence intervals. By adopting certain preventive measures, the experimental errors were substantially reduced.

As a result of analysis of errors some modifications were made in both adsorption and desorption experiments. The P adsorption and desorption curves obtained with improved technique were better than the previous ones. By plotting the adsorption and desorption isotherms on one graph, it was observed that the desorption isotherm occurred above the adsorption isotherm in all the three soils. The adsorption and desorption lines did not meet at the top end as they should. It was thought that either P continued to be adsorbed or some microbial P uptake had taken place with passage of time.

To find the effect of time of shaking on P adsorption and desorption some experiments were carried out. In the P adsorption experiment, it was observed that there was significant decrease in equilibrium P concentration with the increasing time of shaking. The decrease in equilibrium P concentration was rapid before the first measurement was made after six hours and then slowed down with time. In the P desorption experiment there was no significant instantaneous P desorption either in low dilution or high dilution. The trend of time with P desorption was only significant in one soil, whereas in other soils no significant trend of P desorption.

The loss of P in final equilibrium solution lead to test another possibility of microbial P uptake. To avoid

microbial P uptake the effectiveness of some soil sterilants were tried. The P adsorption experiment was carried out on six treatments including control. But the results were inconclusive as there was significant increase in equilibrium P concentration due to various treatments. However, there was no significant increase in case of sodium azide and toluene. To select an appropriate soil sterilant, 0.5M acetic acid extractable P was measured in all treatments. It was observed that the extractable P was increased significantly by autoclaving, τ-irradiation and chloroform treatments over control. It indicated that P status changed with these treatments. It was inferred from the results that either sodium azide or toluene may be appropriate soil sterilant for P adsorption-desorption study. As some problems with the use of sodium azide have been pointed out by some workers the use of toluene was preferred. Toluene as microbial inhibitor has been used by many workers in the P adsorption studies. In the P adsorption experiment for 72 hours time of shaking, the effect of toluene was significant in one soil.

7.4 The effect of plant growth on P in soils

The effect of ryegrass planting on P in soils were investigated in a pot experiment under controlled conditions. The results showed significantly highest dry matter(tops) yield in Darvel A and Midelney (grass) soils

and P content of leaves was highest in the Midelney soil (Table 6.1). The P uptake was increased with increasing P content in leaves (Fig. 6.2).

The $NaHCO_3$ extractable P before planting was significantly correlated with the dry matter (tops) yield ($r = 0.90^*$) and with P uptake ($r = 0.89^*$). The P uptake in leaves was also significantly correlated ($r = 0.91^*$) with the dry matter yield. The extractable P by 3 extractants in grassed soils was significantly reduced than nongrassed soils and in some soils before planting. The effect of plant growth was observed on P adsorption isotherms. The regression analysis of the Langmuir equations obtained from P adsorbed before planting, non-grassed and grassed soils showed significant differences only in Midelney (Grass) and Dreghorn A soils. The plant growth also revealed variable effects on enzymic activities in before planting, grassed and non-grassed soils.

It is evident from the results that sodium bicarbonate extractable P was relatively less in soils produced less dry matter yield. Hence it may be regarded as the index of plant avaialble P in soils. The enzyme activitities may also contributed towards the increase in crop yield in some soils.

7.5 Further development of this work

Phosphate is thoroughly studied plant nutrient element in agriculture. Due to its complex behaviour in soils many aspects still remain to be studied. The present results and the reports in literature about the behaviour of phosphate in soils are conflicting. The reasons for this inconsistency are the tribasic nature of phosphate, strong interaction with many inorganic and organic solid phases, continual uptake by plants and microorganisms, continual return from organic decay and slow reacton rates. Therefore much work is needed to unveil the factors responsible for unavailability of phosphate to plants.

Phosphate adsorption isotherm is useful tool for evaluating the phosphorus fertility status and phosphorus fertilizer requirement of a soil. But this technique has not been adopted for routine soil testing. In the present study P adsorption behaviour of various soils was compared. Still more study is needed for better understanding of the phosphate adsorption mechanism and the identification of reactive sites responsible for various processes associated with the phenomenon. The effects of some amendments like liming and manure on P adsorption is also a subject of future study.

The P desorption with deionized water was also studied. There was no evidence of P desorption with deionized water. Further study in this respect with the use of some electrolytes is needed.

REFERENCES

Adams, J.F. and J.W. Odom (1985). Effects of pH and phosphorus rates on soil-solution phosphorus and phosphorus availability. Soil Science. 140:202-205.

Adams, W.A., S.N. Gafoor and M.I. Karim (1987). Composition and properties of poorly ordered minerals in Welsh soils. II. Phosphate adsorption and reactivity towards NaF solution. Journal of Soil Science. 38:95-103.

Adamson, A.W. (1960). Physical Chemistry of Surfaces. Wiley and Sons (Inter-science Publishers), New York pp. 470-471.

Ahenkorah, Y. (1968). Phosphorus retention capacities of some cocoa-growing soils of Ghana and their relationship with soil properties. Soil Science. 105:24-30.

Amer, F., D.R. Bouldin, C.A. Black and F.R. Duke (1955). Characterization of soil phosphorus by anion exchange resin adsorption and P32 equilibration. Plant and soil. 6:391-408.

Aminuddin, H., J. Vanderdeelen and L. Baert (1987). Extractability of residual rock and superphosphate on limed and unlimed Bungor soil. Pertanika. 10:31-35.

Appelt, H., N.T. Coleman and P.F. Pratt (1975). Interactions between organic compounds, minerals and ions in volcanic-ash-derived soils. II. Effects of organic compounds on the adsorption of phosphate. Soil Science Society of America Proceedings. 39:628-630.

Archer, J. (1988). CROP NUTRITION AND FERTILISER USE. Farming Press Ltd pp. 57-64.

Aura, E. (1978). Determination of available soil phosphorus by chemical methods. Journal of the Scientific Agricultural Society of Finland. 50:305-316.

Bache, B.W. (1964). Aluminum and iron phosphate studies relating to soils. II. Reactions between phosphate and hydrous oxides. Journal of Soil Science. 15:110-116.

Bache, B.W. and C. Ireland (1980). Desorption of phosphate from soils using anion exchange resins. Journal of Soil Science. 31:297-306.

Bache, B.W. and N.E. Rogers (1970). Soil phosphatic values in relation to phosphate supply to plants from some Nigerian soils. Journal of Agri. Sci. (Cambridge). 74:383-390.

Bache, B.W. and E.G. Williams (1971). A phosphate sorption index for soils. Journal of Soil Science. 22:289-301.

Baifan, J. and G. Yichu (1989). A suggested fractionation scheme of inorganic phosphorus in calcareous soils. Fertilizer Research. 20:159-165.

Bakhsh, A. (1988) Studies on the chemistry and behaviour of zinc in soil. Ph.D. Thesis, University of Glasgow.

Barber, S.A. (1962). A diffusion and mass flow concept of soil nutrient availability. Soil Science. 93:39-49.

Barrow, J. (1989). Surface reactions of phosphate in soil. Agricultural Science. 2:33-37.

Barrow, N.J. (1974). Effect of previous additions of phosphate on phosphate adsorption by soils. Soil Science. 118:82-89.

Barrow, N.J. (1967). Relationship between uptake of phosphorus by plants and the phosphorus potential and buffering capacity of the soil-An attempt to test Schofield's hypothesis. Soil Science. 104:99-106.

Barrow, N.J. (1979). The description of desorption of phosphate from soil. Journal of Soil Science. 30:259-270.

Barrow, N.J. (1983b). A mechanistic model for describing the sorption and desorption of phosphate by soil. Journal of Soil Science. 34:733-750.

Barrow, N.J. (1983a). A discussion of the methods for measuring the rate of reaction between soil and phosphate. Fertilizer Research. 4:51-61.

Barrow, N.J. (1983c). On the reversibility of phosphate sorption by soils. Journal of Soil Science. 34:751-758.

Barrow, N.J. (1980). Evaluation and utilization of residual phosphorus in soils. In: The Role of Phosphorus in Agriculture. ASA-CSSA-SSSA, Madison, USA pp. 333-359.

Barrow, N.J. (1970). Comparison of the adsorption of molybdate, sulphate and phosphate by soils. Soil Science. 109:282-288.

Barrow, N.J. (1978). The description of phosphate adsorption curves. Journal of Soil Science. 29:447-462.

Barrow, N.J., L. Madrid and A.M. Posner (1981). A partial model for the rate of adsorption and desorption of phosphate by goethite. Journal of Soil Science. 32:399-407.

Barrow, N.J. and T.C. Shaw (1975). The slow reaction between soil and anions: 2, 3 and 5. Soil Science. 119:167-320.

Barrow, N.J. and T.C. Shaw (1976). Sodium bicarbonate as an extractant for soil phosphate. I.Separation of the factors affecting the amount of phosphate displaced from soil from those affecting secondary adsorption. Geoderma. 16:91-107.

Bates, T.E. (1990). Prediction of phosphorus availability from 88 Ontario soils using five phosphorus soil tests. Communications in Soil Science and Plant Analysis. 21(13-16):1009-1023.

Beckett, P.H.T. and R.E. White (1964). Studies on the phosphate potentials of soils. Part III. The pool of labile inorganic phosphate. Plant and soil. 21:253-282.

Beckwith, R.S. (1964). Sorbed phosphate at standard supernatant concentration as an estimate of the phosphate needs of soils. Australian Journal of Exp. Agr. and Animal Husbandry. 5:52-58.

Birch, H.F. (1961). Phosphorus transformations during plant decomposition. Plant and soil. 15:347-366.

Blakemore, L.C., P.L. Searle and B.K. Daly (1987). Methods of Chemical Analysis of Soils. New Zealand Soil Bureau Scientific Report 80. pp. 103.

Bohn, H.L., B.L. McNeal and G.A. O'Connor (1985). In: Soil Chemistry. John Wiley and sons inc., New York.

Bolan, N.S., N.J. Barrow and A.M. Posner (1985). Describing the effect of time on sorption of phosphate by iron and aluminium hydroxides. Journal of Soil Science. 36:187-197.

Bower, C.A., J.T. Hatcher (1967). Adsorption of fluoride by soils and minerals. Soil Science. 103: 151-154.

Bowman, R.A. and C.V. Cole (1978). Transformation of organic phosphorus substances in soil evaluated by sodium bicarbonate extraction. Soil Science. 125:49-55.

Bray, R.H. and L.T. Kurtz (1945). Determination of total, organic, and available forms of phosphorus in soils. Soil Science. 59:39-45.

Breeuwsma, A., J.H.M. Wosten, J.J. Vleeshouwer, A.M. van Slobbe and J. Bouma (1986). Derivation of land qualities to assess environmental problems from soil surveys. Soil Science Society of America Journal. 50:186-190.

Brewster, J.L., A.N. Gancheva and P.H. Nye (1975). The determination of desorption isotherm for soil phosphate using low volumes of solution and an anion exchange resin. Journal of Soil Science. 26:364-377.

Brookes, P.C., D.S. Powlson and D.S. Jenkinson (1982). Measurement of microbial biomass phosphorus in soil. Soil Biology and Biochemistry. 14:319-329.

Browman, M.G. and M.A. Tabatabai (1978). Phosphodiesterase activity of soils. Soil Science Society of America Journal. 42:284-290.

Campbell, L.B. and G.J. Racz (1975). Organic and inorganic phosphorus content, movement and mineralization of phosphorus in soil beneath a feedlot. Canadian Journal of Soil Science. 55:457-466.

Cathcart, J.B. (1980). World phosphate reserves and resources In: The Role of Phosphorus in Agriculture. ASA-CSSA-SSSA, Madison, USA pp. 1-17.

Cerna, S. (1972). Phosphatase activity in structural soil. Acta University Caol. Biologica. 1970:455-459.

Chakravarti, S.N. and O. Talibudeen (1962). Phosphate equilibria in acid soils. Journal of Soil Science. 13:231-240.

Chang, S.C. and M.L. Jackson (1957). Fractionation of soil phosphorus. Soil Science. 84:133-144.

Chang, S.C. and S.R. Juo (1963). Available phosphorus in relation to forms of phosphates in soils. Soil Science. 95:91-96.

Chauhan, C.S., J.W.B. Stewart and E.A. Paul (1979). Effect of carbon addition on soil labile inorganic, organic and microbially held phosphate. Canadian Journal of Soil Science. 59:387-396.

Chien, S.H. (1978). Interpretation of Bray 1 extractable phosphorus from acid soils treated with phosphate rocks. Soil Science. 126:34-39.

Cline, G.R., W.C. Lindemann and R. Quintero (1985). Dynamics of extractable phosphorus during non sterile and sterile incubation of sludge amended soil. Soil Science. 140:98-104.

Cole, C.V., E.T. Elliott, H.W. Hunt and D.C. Coleman (1978). Trophic interactions in soils as they affect energy and nutrient dynamics. V. Phosphorus transformations. Microbial Ecology. 4:381-387.

Cole, C.V., S.R. Olsen and C.O. Scott (1953). The nature of phosphate sorption by calcium carbonate. Soil Science Society of America Proceedings. 17:352-356.

Colwell, J.D. (1963). The estimation of phosphorus fertilizer requirements of wheat in southern New Wales by soil analysis. Australian Journal of Exp. Agric. Anim. Husb.. 3:190-197.

Colwell, J.D. (1965). Determination of phosphorus in sodium hydrogen carbonate extracts of soils. Chemistry and Industry. 21:893-895.

Cooke, I.J. and J. Hislop (1963). Use of anion exchange resin for the assessment of available soil phosphate. Soil Science. 95:308-312.

Crisanto, T. and C.D. Sutton (1973). Measurement of available phosphate content of some Spanish soils. Plant and soil. 39:399-412.

Cuttle, S.P. (1983). Chemical properties of upland peats influencing the retention of phosphate and potassium ions. Journal of Soil Science. 34:75-82.

Dalal, R.C. (1985). Comparative prediction of yield response and phosphorus uptake from soil using anion- and cation-anion-exchange resins. Soil Science. 139:227-231.

Dyer, B. (1894). On the analytical determination of probable available "mineral" plant food in soils. Journal of the Chemical Society. Transactions. 65:115-167.

Eivazi, F. and M.A. Tabatabai (1977). Phosphatases in soils. Soil Biology and Biochemistry. 9:167-172.

Ekpete, D.M. (1976). Evaluation of chemical methods for the determination of available phosphorus in waterlogged soils. Soil Science. 121:217-221.

Elrashidi, M.A. and S. Larsen (1978). The effect of phosphate addition on the solubility of phosphate in soil. Plant and Soil. 50:585-596.

Enfield, C.G., T. Phan, D.M. Walters and R.J. Ellis (1981). Kinetic model for phosphate transport and transformation in calcareous soils I and II. Soil Science Society of America Journal. 45:1059-1070.

Enwezor, W.O. (1977). Soil testing for phosphorus in some Nigerian soils: I. Comparison of methods of determining available phosphorus in soils of Southeastern Nigeria. Soil Science. 123:48-53.

Fardeau, J.C., C. Morel and R. Boniface (1988). Why the Olsen method should be used to estimate <<available soil phosphorus>>(Fr.). Agronomie. 8:577-584.

Fiskell, J.G.A., R.S. Mansell, H.M. Selim and F.G. Martin (1979). Kinetic behavior of phosphate sorption by acid sandy soil. Journal of Environmental Quality. 8:579-584.

Fitter, A.H. (1992). Roots. In: The Soil. Harper-Collins Publishers pp. 49-54.

Fitter, A.H. and C.D. Sutton (1975). The use of the Freundlich isotherm for soil phosphate sorption data. Journal of Soil Science. 26:241-246.

Fitts, J.W. and W.L. Nelson (1956). The determination of lime and fertilizer requirements of soils through chemical tests. Advances in Agronomy. 8:241-282.

Fox, R.L. (1981/1982). Using phosphate sorption curves to determine P(Phosphorus) fertilizer requirements. Better Crops Plant Food. 66:24-29.

Fox, R.L. and E.J. Kamprath (1970). Phosphate sorption isotherms for evaluating the phosphate requirements of soils. Soil Science Society of America Proceedings. 34:902-907.

Fox, R.L., W.M.H. Saunders and S.S.S. Rajan (1986). Phosphorus nutrition of pasture species: Phosphorus requirement and root saturation values. Soil Science Society of America Journal. 50:142-148.

Fox, R.L. and L. Xue-yuan (1986). Phosphate fertilizer requirements of weathered soils and residual phosphate fertilizer efficiency as indicated by phosphate sorption curves. Proceedings of the International Symposium on Red soils. 468-478.

Freeman, J.S. and D.L. Rowell (1981). The adsorption and precipitation of phosphate onto calcite. Journal of Soil Science. 32:75-84.

Gama, B.M., B.R. Singh and A.P. Uriyo (1987). Phosphate sorption and desorption in Tanzanian Haplustox and Vitrandept soils. East Afr. Agric. For. J. 52:213-220.

Gilliam, J.W. (1970). Hydrolysis and uptake of pyrophosphate by plant roots. Soil Science Society of America Proceedings. 34:83-86.

Giroux, M. and T.S.e. Tran (1985). Evaluation of different available P extracting methods in relation to oat yield and soil properties. Canadian Journal of Soil Science. 65:47-60.

Goldberg, S. and G. Sposito (1984). A chemical model of phosphate adsorption by soils: I.Reference oxide minerals. Soil Science Society of America Journal. 48:772-778.

Gomez, K.A. and A.A. Gomez (1984). Statistical Procedure for Agricultural Research. 2nd edition. John Wiley and sons inc., New York pp. 1-240.

Grant, R., C.J. Bown and E.L. Birse (1962). Map sheet 14, Ayr. Soil Survey of Scotland. The Macualay Institute for Soil Research, Aberdeen.

Grava, J. (1975). Causes for variation in phosphorus soil tests. Communications in Soil Science and Plant Analysis. 6:129-138.

Greaves, M.P. and D.M. Webley (1965). A study of the breakdown of organic phosphate by microorganisms from the root region of certain pasture grasses. Journal of Applied Bacteriology. 28:454-465.

Griffin, G.F. (1971). Effect of liming on the soil test level of phosphorus as determined by three methods. Soil Science Society of America Proceedings. 35:540-542.

Griffin, R.A. and J.J. Jurinak (1973). The interaction of phosphate with calcite. Soil Science Society of America Proceedings. 37:847-850.

Gunary, D. (1970). A new adsorption isotherm for phosphate in soil. Journal of Soil Science. 21:72-77.

Gunary, D. and C.D. Sutton (1967). Soil factors affecting plant uptake of phosphate. Journal of Soil Science. 18:168-173.

Gupta, M.B.S. and A.H. Cornfield (1963). Phosphorus in calcareous soils. III. 'Available' phosphate in calcareous soils as measured by five chemical methods and phosphate uptake by Rye-grass in a pot test. Journal of Science Food and Agriculture. 14:563-567.

Halm, B.J., J.W.B. Stewart and R.L. Halstead (1972). The phosphorus cycle in a native-grassland ecosystem. In: Isotopes and Radiation in Soil Plant Relationships Including Forestry. Palmerstone North, New Zealand pp. 571-586.

Harter, R.D. (1969). Phosphorus adsorption sites in soils. Soil Science Society of America Proceedings. 33:630-632.

Harter, R.D. (1968). Adsorption of phosphorus by lake sediments. Soil Science Society of America Proceedings. 32:514-518.

Harter, R.D. and D.E. Baker (1977). Applications and misapplications of the Langmuir equation to soil adsorption phenomena. Soil Science Society of America Journal. 41:1077-1080.

Hedley, M.J. and J.W.B. Stewart (1982). Method to measure microbial phosphates in soils. Soil Biology and Biochemistry. 14:377-385.

Helyar, K.R., D.N. Munns and R.G. Bureau (1976). Adsorption of phosphate by gibbsite. I. Effects of neutral chloride salts of calcium, magnesium, sodium and potassium. Journal of Soil Science. 27:307-314.

Herbien, S.A. and J.L. Neal (1990a). Phosphatase activity in arctic tundra soils disturbed by vehicles. Soil Biology and Biochemistry. 22:853-858.

Herbien, S.A. and J.L. Neal (1990b). Soil pH and phosphatase activity. Communications in Soil Science and Plant Analysis. 21:439-456.

Hislop, J. and I.J. Cooke (1968). Anion exchange resin as a means of assessing soil phosphate status: A laboratory technique. Soil Science. 105:8-11.

Hodgson, J.M. (1976). Soil Survey Field Handbook. Soil Survey Technical Monograph No. 5, Rothamsted Experimental Station, Harpenden.

Holford, I. (1989). Phosphate behaviour in soil. Agricultural Science. 2:15-21.

Holford, I.C.R. (1976). Effects of phosphate buffer capacity of soil on the phosphate requirements of plants. Plant and soil. 45:433-444.

Holford, I.C.R. (1980). Greenhouse evaluation of four phosphorus soil tests in relation to phosphate sorption and labile phosphate in soils. Soil Science Society of America Proceedings. 44:555-559.

Holford, I.C.R. (1979). Evaluation of soil phosphate buffering indices. Australian Journal of Soil Research. 17:495-504.

Holford, I.C.R. (1982). The comparative significance and utility of the Freundlich and Langmuir parameters for characterizing sorption and plant availability of phosphate in soils. Australian Journal of Soil Research. 20:233-242.

Holford, I.C.R. and B.R. Cullis (1985). An evaluation of eight soil phosphate extractants on acid wheat-growing soils. Australian Journal of Soil Research. 23:647-653.

Holford, I.C.R. and G.E.G. Mattingly (1976a). A model for the behaviour of labile phosphate in soil. Plant and soil. 44:219-229.

Holford, I.C.R. and G.E.G. Mattingly (1976b). Phosphate adsorption and plant availability of phosphate. Plant and soil. 44:377-389.

Holford, I.C.R. and G.E.G. Mattingly (1975). The high- and low-energy phosphate adsorbing surfaces in calcarious soils. Journal of Soil Science. 26:407-417.

Holford, I.C.R., R.W.M. Wedderburn and G.E.G. Mattingly (1974). A Langmuir two-surface equation as a model for phosphate adsorption by soils. Journal of Soil Science. 25:242-255.

Holford, I.C.R. and G.J. Crocker (1988). Efficacy of various soil phosphate tests for predicting phosphate responsiveness and requirements of clover pastures on acidic tableland soils. Australian Journal of Soil Research. 26:479-488.

Hons, F.M., L.A. Larson-Vollmer and M.A. Locke (1990). NH4OAc-EDTA extractable phosphorus as a soil test procedure. Soil Science. 149:249-256.

Hooker, M.L., G.A. Peterson, D.H. Sander and L.A. Daigger (1980). Phosphate fractions in calcareous soils as altered by time and amounts of added phosphate. Soil Science Society of America Journal. 44:269-277.

Hope, G.D. and J.K. Syers (1976). Effects of solution:soil ratio on phosphate sorption by soils. Journal of Soil Science. 27:301-306.

Horneck, D.A., J.M. Hart and D.C. Peek (1990). The influence of sampling intensity, liming, P rate and method of P application on P soil test values. Communications in Soil Science and Plant Analysis. 21:1079-1090.

Huang, C.P. (1975). Adsorption of phosphate at the hydrous τ-Al_2O_3. Journal of Colloid Interface Science. 53:175-186.

Imai, H., K.W.T. Goulding and O. Talibudeen (1981). Phosphate adsorption in allophanic soils. Journal of Soil Science. 32:555-570.

Ipinmidun, W.P. (1973). Assessment of residues of phosphate application in some soils of Northern Nigeria I. Examination of L and E values. Plant and soil. 39:213-225.

Jackson, R.W. and J.A. De Moss (1965). Effect of toluene on Escherichia coli. Journal of Bacteriology. 90:1420-1425.

Jackson, T.L., S. Vacharotayan, L.A. Alban and R. Peterson (1964). Phosphorus availability in reddish brown laterite soils. II. Relationships between field, greenhouse and soil analyses. Agronomy Journal. 56:558-560.

Jakobsen, I. and A.J. Andersen (1982). Vesicular-arbuscular mycorrhiza and growth in barley: Effects of irradiation and heating of soil. Soil Biology and Biochemistry. 14:171-178.

John, M.K. (1972). Extractable phosphorus related to forms of phosphorus and their soil properties. Journal of Science Food and Agriculture. 23:1425-1433.

Jones, J.B.J. (1990). Universal soil extractants: their composition and use. Communications in Soil Science and Plant Analysis. 21:1091-1101.

Juma, N.G. and M.A. Tabatabai (1978). Distribution of phosphomonoesterases in soils. Soil Science. 126:101-108.

Juo, A.S.R. and R.L. Fox (1977). Phosphate sorption characteristics of some benchmark of West Africa. Soil Science. 124:370-376.

Kafkafi, U., A.M. Posner and J.P. Quirk (1967). Desorption of phosphate from Kaolinite. Soil Science Society of America Proceedings. 31:348-353.

Kamprath, E.J. and M.E. Watson (1980). Conventional soil and tissue tests for assessing the phosphorus status of soils. In: The Role of Phosphorus in Agriculture. ASA-CSSA-SSSA, Madison, USA pp. 433-469.

Kato, N. and N. Owa (1989). Kinetics of phosphate adsorption by sandy and clayey soils. Soil Science and Plant Nutrition. 35:119-129.

Kelley, W.D. and R. Rodriguez-Kabana (1979). Effects of sodium azide and methyl bromide on soil bacterial populations, enzymic activities and other biological variables. Pesticide Science 10:207-215.

Kepert, D.G., A.D. Robson and A.M. Posner (1979). The effect of organic root products on the availability of phosphorus to plants. In: The Soil-Root Interphase. Academic Press London pp. 116-124.

Khalid, R.A., W.H. Patrick and R.D. Delaune (1977). Phosphorus sorption characteristics of flooded soils. Soil Science Society of America Journal. 41:305-310.

Khalil, N.F. (1981). Investigation of inorganic factors in soil limiting the growth of Sitka Spruce, Ph.D. Thesis, University of Glasgow.

Khan, M.Q. (1987). Studies on the measurement of extractable and mineralizable nitrogen in soil. Ph.D. Thesis, University of Glasgow.

Khasawneh, F.E. (1971). Solution ion activity and plant growth. Soil Science Society of America Proceedings. 35:426-436.

Kiss, S., M. Dracan-Bularda and D. Radulescu (1975). Biological significance of enzymes accumulated in soil. Advances in Agronomy. 27:25-87.

Kuo, S., E.J. Jellum and W.L. Pan (1988). Influence of phosphate sorption parameters of soils on the desorption of phosphate by various extractants. Soil Science Society of America Journal. 52:974-979.

Kuo, S. and E.G. Lotse (1972). Kinetics of phosphate adsorption by calcium carbonate and Ca-kaolinite. Soil Science Society of America Proceedings. 36:725-729.

Kuo, S. and E.G. Lotse (1974). Kinetics of phosphate adsorption and desorption by hematite and gibbsite. Soil Science. 116:400-406.

Kurtz, L.T. and J.P. Quirk (1965). Phosphate adsorption and phosphate fractions in field soils of varying histories of phosphate fertilization. Australian Journal of Agricultural Research. 16:403-412.

LaFleur, K.S. and G.R. Craddock (1967). Use of metalizable dyes to minimize the phosphate sorption of Cecil soil. Soil Science Society of America Proceedings. 31:324-327.

Lajtha, K. and S.H. Bloomer (1988). Factors affecting phosphate sorption and phosphate retention in a desert ecosystem. Soil Science. 146:160-167.

Langmuir, I. (1918). The adsorption of gases on plane surfaces of glass, mica and platinum. Journal of American Chem. Society. 40:1361-1402.

Larsen, S. (1967). Soil phosphorus. Advances in Agronomy. 19:151-210.

Larsen, S., D. Gunary and C.D. Sutton (1965). The rate of immobilization of applied phosphate in relation to soil properties. Journal of Soil Science. 16:141 - 148.

Le Mare, P.H. (1981). Phosphorus sorption and release. In: Characterization of Soils in Relation to their Classification and Management for Crop Production: Examples from Some Areas of the Humid Tropics. Oxford University Press.

Leaver, J.P. and E.W. Russell (1957). The reaction between phosphate and phosphate fixing soils. Journal of Soil Science. 8:113-126.

Lee, J.D. and T.D. Lee (1983). Errors In: Statistics and Computer Methods in Basic. Van Nostrand Reinhold (UK) Co. Ltd. pp. 4-12.

Lennox, L.J. (1979). An automated procedure for the determination of phosphorus. Water Research. 13:1329-1333.

Lewis, D.G. and J.P. Quirk (1967). Phosphate diffusion in soil and uptake by plants. III and IV. Plant and soil. 26:445-468.

Lindsay, W.L. (1979). In: Chemical Equilibria in Soils. John Wiley and sons inc., New York.

Lindsay, W.L. and E.C. Moreno (1960). Phosphate phase equilibria in soils. Soil Science Society of America Proceedings. 24:177-182.

Loganathan, P., N.O. Isirimah and D.A. Nwachuku (1987). Phosphorus sorption by ultisols and inceptisols of the Niger delta in southern Nigeria. Soil Science. 144:330-338.

Low, P.F. and C.A. Black (1950). Reactions of phosphate with kaolinite. Soil Science. 70:273-290.

Luscombe, P.C., J.K. Syers and P.E.H. Gregg (1979). Water extraction as a soil testing procedure for phosphate. Communications in Soil Science and Plant Analysis. 10:1361-1370.

Madrid, L. and P.d. Arambarri (1985). Adsorption of phosphate by two iron oxides in relation to their porosity. Journal of Soil Science. 36:523-530.

Madrid, L. and A.M. Posner (1979). Desorption of phosphate from goethite. Journal of Soil Science. 30:697-707.

MAFF (1983). Fertilizer recommendations for agricultural and horticultural crops. Ministry of Agriculture, Fisheries and Food, Bulletin. Number 209

MAFF (1986). The Analysis of Agricultural Materials. Reference Book 427, 3rd edition. Ministry of Agriculture, Fisheries and Food. HMSO, London.

Manikandan, P. and T.J. Sastry (1986). Desorption of adsorbed phosphate in a few soils belonging to Alfisols, Inceptisols and Entisols from Mysore Plateau. Journal of the Indian Society of Soil Science. 34:749-756.

Martin, J.K. (1973). The influence of rhizosphere microflora on the availability of ^{32}P-myoinositol hexaphosphate phosphorus to wheat. Soil Biology and Biochemistry. 5:473-483.

Mathur, S.P. and A.F. Rayment (1977). Influence of trace element fertilizer on the decomposition rate and phosphatase activity in soils. Canadian Journal of Soil Science. 57:397-408.

Mattingly, G.E.G. (1971). Residual value of phosphate fertilizer on neutral and calcareous ground. In: Residual Value of Applied Nutrients. Tech. Bull. 20 : 1-9. Ministry of Agriculture Fisheries and Food, HMSO, London.

Mattingly, G.E.G. and A. Pinkerton (1961). Some relationships between isotopically exchangeable phosphate, soil analysis and crop growth in the greenhouse. Journal of Science Food and Agriculture. 12:772-777.

Mattingly, G.E.G., R.D. Russell and B.M. Jephcott (1963). Experiments on cumulative dressings of fertilizers in calcareous soils in South-West England: II. Phosphorus uptake by ryegrass in the greenhouse. Journal of Science Food and Agriculture. 14:629-637.

Mays, D.A., S.R. Wilkinson and C.V. Cole (1980). Phosphorus nutrition of forages. In: The Role of Phosphorus in Agriculture. ASA-CSSA-SSSA, Madison, USA pp. 805-843.

Mazumder, A.R. (1992). Effects of pesticides on soil microbiological processes. M.Sc. Thesis, University of Glasgow, UK.

McLachlan, K.D. (1976). Comparative phosphorus responses in plants to a range of available phosphorus situations. Australian Journal of Agricultural Research. 27:323-341.

McLaughlin, M.J., A.M. Alston and J.K. Martin (1986). Measurement of phosphorus in the microbial biomass: A modified procedure for field soils. Soil Biology and Biochemistry. 18:437-443.

Mehta, B.V. and J.M. Patel (1963). Some aspects of phosphorus availability in Gujrat soils. Journal of the Indian Society of Soil Science. 11:151-158.

Mendoza, R.E. (1989). Different performances of soil phosphate tests for reflecting the effects of buffering capacity on uptake of native phosphate with time. Plant and Soil. 113:13-19.

Mendoza, R.E. and N.J. Barrow (1987). Ability of three soil extractants to reflect the factors that determine the availability of soil phosphate. Soil Science. 144:319-329.

Mendoza, R.E., A. Canduci and C. Aprile (1990). Phosphate release from fertilized soils and its effect on the changes of phosphate concentration in soil solution. Fertilizer Research. 23:165-172.

Mengel, K. and E.A. Kirkby (1982). Principles of plant nutrition. (3rd Edition). International Potash Institute, Bern, Switzerland pp. 355.

Menon, R.G., S.H. Chien and L.L. Hammond (1990). Development and evaluation of the Pi soil test for plant-available phosphorus. Communications in Soil Science and Plant Analysis. 21:1131-1150.

Menon, R.G., S.H. Chien and L.L. Hammond (1989). Comparison of Bray-I and Pi tests for evaluating plant-available phosphorus from soils treated with different partially acidulated phosphate rocks. Plant and soil. 114:211-216.

Mitchell, B.D. and R.A. Jarvis (1956). The soils of the country round Kilmarnock. Memoirs of the Soil Survey of Great Britain. Soil Survey of Scotland. The Macualay Institute for Soil Research, Aberdeen.

Moore, C.S. and G.S.P. Ritchie (1988). Aluminium speciation and pH of an acid soil in the presence of fluoride. Journal of Soil Science. 39:1-8.

Mott, C.J.B. (1981). Anion and ligand exchange. In: The Chemistry of Soil Processes. John Wiley & Sons Ltd. pp. 179-216.

Muljadi, D., A.M. Posner and J.P. Quirk (1966). The mechanism of phosphate adsorption by kaolinite, gibbsite and pseudobohemite. I, II and III. Journal of Soil Science. 17:212-247.

Munns, D.N. and R.L. Fox (1976). The slow reaction which continues after phosphate adsorption: kinetics and equilibrium in some tropical soils. Soil Science Society of America Proceedings. 40:46-51.

Murphy, J. and J.P. Riley (1962). A modified single solution method for the determination of phosphate in natural waters. Analytical Chemica Acta. 27:31-36.

Naidu, R., R.W. Tillman, J.K. Syers and J.H. Kirkman (1987). Effect of liming on phosphate extracted by two soil-testing procedures. Fertilizer Research. 14:143-152.

Nair, K.P.P. and K. Mengel (1984). Importance of phosphate buffer power for phosphate uptake by rye. Soil Science Society of America Journal. 48:92 -95.

Nelson, W.L., A. Mehlich and E. Winters (1953). The development, evaluation and use of soil tests for phosphorus availability. In: W.H. Pierre and A.G. Norman (ed.) Soil and Fertilizer Phosphorus. Agronomy. 4:153-158.

Ng, S.K. (1970). Automated determination of soils under rubber cultivation. Journal of Science Food and Agriculture. 21:275-278.

Olsen, S.R., C.V. Cole, F.S. Watanabe and L.A. Dean (1954). Estimation of available phosphorus in soils by extraction with sodium bicarbonate. USDA Circular. 939

Olsen, S.R. and F.E. Khasawneh (1980). Use and limitations of physical-chemical criteria for assessing the status of phosphorus in soils. In: The Role of Phosphorus in Agriculture. ASA-CSSA-SSSA, 677 South Segoe Road, Madison, WI 53711, USA.

Olsen, S.R. and L.E. Sommers (1982). Phosphorus In: Methods of Soil Analysis, Part 2. Chemical and Microbiological Properties-Agronomy Monograph No. 9. ASA-SSSA, Madison, USA pp. 403-430.

Olsen, S.R. and F.S. Watanabe (1963). Diffusion of phosphorus as related to soil texture and plant uptake. Soil Science Society of America Proceedings. 27:648-653.

Olsen, S.R. and F.S. Watanabe (1957). A method to determine a phosphorus adsorption maximum of soils as measured by the Langmuir isotherm. Soil Science Society of America Proceedings. 21:144-149.

Oskay, K.S. (1986). The influence of time on phosphate retention and the description of P adsorption by the means of Langmuir isotherms in calcareous soils. Doga Tr. Journal of Agriculture and Forestry. 10:252-262.

Ozanne, P.G. and T.C. Shaw (1968). Advantages of the recently developed phosphate sorption test over the older extractant methods for soil phosphate. Trans. 9th International Congr. Soil Science (Adelaide, Australia). 2:273-280.

Ozanne, P.G. and T.C. Shaw (1967). Phosphate sorption by soils as a measure of the phosphate requirement for pasture growth. Australian Journal of Agricultural Research. 18:601-612.

Paauw, F.V.d. (1971). An effective water extraction method for the determination of plant available phosphorus. Plant and Soil. 34:467-481.

Palacios Fernandez, D.F. and O.G. Us'Yarov (1990). Comparison of models of phosphate sorption by soils. Soviet Soil Science. 22:66-71.

Parfitt, R.L. (1978). Anion adsorption by soils and soil materials. Advances in Agronomy. 30:1-50.

Patiram, R.N.R. and R.N. Prasad (1990). Forms of soil phosphorus and suitable extractants for available phosphorus in acid soils of Sikkim. Journal of the Indian Society of Soil Science. 38:237-242.

Peech, M. and L. English (1944). Rapid microchemical soil tests. Soil Science. 57:167-195.

Posner, A.M. and J.W. Bowden (1980). Adsorption isotherms: should they be split. Journal of Soil Science. 31:1-10.

Prutton, C.F. and S.H. Maron (1953). In: Fundamental Principles of Physical Chemistry. Macmillan Company, New York.

Pulford, I.D. and H.J. Duncan (1975). The influence of pyrite oxidation products on the adsorption of phosphate by coal-mine waste. Journal of Soil Science. 26:74-80.

Radwan, M.A., J.M. Kraft and J.S. Shumway (1985). Evaluation of different extractants for phosphorus in Western Hemlock soils. Soil Science. 140:429-435.

Ragg, J.M., B.M. Shipley, N.A. Duncan, J.S. Bibby and D.W. Merrilees (1976). Map sheet 31, Airdrie. Soil Survey of Scotland. The Macualay Institute for Soil Research, Aberdeen.

Rajan, S.S.S. (1973). Phosphorus adsorption characteristics of Hawaiian soils and their relationships to equilibrium phosphorus concentrations required for maximum growth of millet. Plant and soil. 39:519-532.

Rajan, S.S.S. and R.L. Fox (1975). Phosphate adsorption by soils. II. Reactions in tropical acid soils. Soil Science Society of America Proceedings. 39:846-851.

Rajan, S.S.S., K.W. Perrott and W.M.H. Saunders (1974). Identification of phosphate-reactive sites of hydrous alumina from proton consumption during phosphate adsorption at constant pH values. Journal of Soil Science. 25:438-447.

Randall, G.W. and J. Grava (1971). Effect of soil: Bray no.1 ratios on the amount of phosphorus extracted from calcareous Minnesota soils. Soil Science Society of America Proceedings. 35:112-114.

Reith, J.W.S., R.H.E. Inkson, N.M. Scott, K.S. Caldwell, J.A.M. Ross and W.E. Simpson (1987). Estimates of soil phosphorus for different soil series. Fertilizer Research. 11:123-142.

Rennie, D.A. and R.B. McKercher (1959). Adsorption of phosphorus by four Saskatchewan soils. Canadian Journal of Soil Science. 39:64-75.

Rozycki, M. and R. Bartha (1981). Problems associated with the use of azide as an inhibitor of microbial activity in soil. Applied and Environmental Microbiology. 41:833-836.

Russell, E.J. and J.A. Prescott (1916). The reaction between dilute acids and the phosphorus compounds of the soils. Journal of Agri. Sci. (Cambridge). 8:65-110.

Ryden, J.C., J.R. McLaughlin and J.K. Syers (1977a). Mechanisms of phosphate sorption by soils and hydrous ferric oxide gel. Journal of Soil Science. 28:72-92.

Ryden, J.C., J.R. McLaughlin and J.K. Syers (1977b). Time dependent sorption of phosphate by soils and hydrous ferric oxides. Journal of Soil Science. 28:585-595.

Ryden, J.C. and J.K. Syers (1975). Rationalization of ionic strength and cation effects on phosphate sorption by soils. Journal of Soil Science. 26:395-406.

Ryden, J.C. and J.K. Syers (1977). Desorption and isotopic exchange relationships of phosphate sorbed by soils and hydrous ferric oxide gel. Journal of Soil Science. 28:596-609.

SAC (1985). Advisory Soil Analysis and Interpretation. Macaulay Institute for Soil research and Scottish Agricultural Colleges pp 1-10.

Salonius, P.D., J.B. Robinson and F.E. Chase (1967). A comparison of autoclaved and gamma-irradiated soils as media for microbial colonisation experiments. Plant and soil. 27:239-248.

Sarirullah, M. (1989). Transformation of phosphorus and its availability to plants in coal mine soils. Ph.D. Thesis, University of Glasgow.

Scaife, M.A. (1968). Maize fertilizer experiments in Western Tanzania. Journal of Agri. Sci. (Cambridge). 70:209-222.

Schofield, R.K. (1955). Can a precise meaning be given to 'available' soil phosphorus?. Soils Fertilizers. 18:373-375.

Seale, R.S. (1975). Soils of the Ely District. Memoirs of the Soil Survey of Great Britain. The Soil Survey Rothamsted Experimental Station, Harpenden.

Shah, S.S.H. (1988). Transformations of nitrogen and its availability to plants in coal mine soils. Ph.D. Thesis, University of Glasgow, UK.

Shailaja, S. and K.L. Sahrawat (1990). Adsorption and desorption of phosphate in some semi-arid tropical Indian vertisols. Fertilizer Research. 23:87-96.

Sharpley, A.N. (1985). Phosphorus cycling in unfertilized and fertilized agricultural soils. Soil Science Society of America Journal. 49:905-911.

Sharpley, A.N. (1991). Soil phosphorus forms extracted by iron-aluminum-oxide impregnated filter paper. Soil Science Society of America Journal. 55:1038 -1041.

Shipp, R.F. and R.P. Matelski (1960). A microscopic determination of apatite and a study of phosphorus in some Nebraska soil profiles. Soil Science Society of America Proceedings. 24:450-452.

Sibbesen, E. (1983). Phosphate soil tests and their suitability to assess the phosphate status of soil. Journal of Science Food and Agriculture. 34:1368-1374.

Sibbesen, E. (1978). An investigation of the Anion-exchange resin method for soil phosphate extraction. Plant and soil. 50:305-321.

Sibbesen, E. (1977). A simple ion exchange resin procedure for extracting plant available elements from soil. Plant and soil. 46:665-669.

Sibbesen, E. (1981). Some new equations to describe phosphate sorption by soils. Journal of Soil Science. 32:67-74.

Sims, J.T. and B.G. Ellis (1983). Adsorption and availability of P following the application of limestone to an acid, aluminous soil. Soil Science Society of America Journal. 47:888-893.

Sissingh, H.A. (1971). Analytical procedure of the PW method, used for the assessment of phosphate status of arable soils in Netherlands. Plant and Soil. 34:483-486.

Skipper, H.D. and D.T. Westermann (1973). Comparative effects of propylene oxide, sodium azide and autoclaving on selected soil properties. Soil Biology and Biochemistry. 5:409-414.

Skujins, J. (1978). History of abiontic soil enzyme research. In: Soil Enzymes. Academic Press, N.Y. pp. 1-49.

Smillie, G.W. and J.K. Syers (1972). Calcium fluoride formation during extraction of calcareous soils with fluoride: II. Implications to the Bray P-1 test. Soil Science Society of America Proceedings. 36:25-30.

Smith, F.W., B.G. Ellis and J. Grava (1957). Use of acid fluoride solutions for the extraction of available phosphorus in calcareous soils and in soils to which rock phosphate has been added. Soil Science Society of America Proceedings. 21:400-404.

Smith, R.G. and P.E.H. Gregg (1982). A comparison of two phosphorus soil tests as inputs to a pasture growth model. New Zealand Journal of Agriculture Research. 25:557-563.

Somani, L.L. (1989). The computation of the plant utilizable phosphorus from capacity, intensity, and kinetic parameters. Polish Journal of Soil Science. 22:27-33.

Speir, T.W. and P.J. Ross (1978). Soil phosphatase and sulphatase. In: Soil Enzymes. Academic Press, N.Y. pp. 197-250.

Stewart, J.W.B., M.J. Hedley and B.S. Chauhan (1980). The immobilisation, mineralisation and redistribution of phosphorus in soils. In: Proceedings of the Western Canada Phosphate Symposium. Alberta Soil Science Society, Emonton pp. 276-306.

Stroo, H.F. and E.M. Jencks (1982). Enzyme activity and respiration in mine soils. Soil Science Society of America Journal. 46:548-553.

Stuanes, A.O. (1984). Phosphorus sorption of soils to be used in wastewater renovation. Journal of Environmental Quality. 13:220-224.

Syers, J.K., M.G. Browman, G.W. Smillie and R.B. Corey (1973). Phosphate sorption by soils evaluated by the Langmuir adsorption equation. Soil Science Society of America Proceedings. 37:358-363.

Syers, J.K., J.T. Murdoch and J.D.H. Williams (1970). Adsorption and desorption of phosphate by soils. Communications in Soil Science and Plant Analysis. 1:57-62.

Syers, J.K., G.W. Smillie and D.H. Williams (1972). Calcium fluoride formation during extraction of calcareous soils with fluoride: I. Implication to inorganic P fractionation schemes. Soil Science Society of America Proceedings. 36:20-25.

Tabatabai, M.A. (1982). Soil enzymes. In: Methods of Soil Analysis, Part 2. Chemical and Microbiological Properties (2nd Edition). ASA-SSSA, Madison, USA pp. 903-947.

Tarafdar, J.C. and A. Jungk (1987). Phosphatase activity in rhizosphere and its relation to the depletion of soil organic phosphorus. Biology and Fertility of Soils. 3:199-204.

Taylor, A.W. and E.L. Gurney (1964). Solubility of variscite. Soil Science. 98:9-13.

Thomas, G.W. and D.E. Peaslee (1973). Testing soils for phosphorus. In: Soil Testing and Plant Analysis. SSSA, Madison, Wisconsin. USA.

Tiarks, A.E. (1982). Phosphorus sorption curves for evaluating phosphorus requirements of loblolly pine (Pinus taeda). Communications in Soil Science and Plant Analysis. 13:619-631.

Tiessen, H.J., W.B. Stewart and C.V. Cole (1984). Pathways of phosphorus transformations in soils of differing pedogenesis. Soil Science Society of America Journal. 48:853-858.

Tinker, P.B. (1980). Role of rhizosphere microorganisms in phosphorus uptake by plants. In: The Role of Phosphorus in Agriculture. ASA-CSSA-SSSA, Madison, USA pp. 617-654.

Tiong, S.M.I. (1987). The incorporation of potassium antimony tartrate in molybdenum blue method for the automated colorimetric determination of available phosphorus in soils. Pertanika. 10:27-29.

Tisdale, S.L., W.L. Nelson and J.D. Beaton (1985). Soil and fertilizer phosphorus. In: Soil Fertility and Fertilizers. Macmillan Publishing Company, New York, U.S.A. pp. 189-248.

Toreu, B.N., F.G. Thomas and G.P. Gillman (1988). Phosphate-sorption characteristics of soils of the North Queensland coastal region. Australian Journal of Soil Research. 26:465-477.

Torrent, J. (1987). Rapid and slow phosphate sorption by Mediterranean soils: effect of iron oxides. Soil Science Society of America Journal. 51:78-82.

Turner, R.C. and H.M. Rice (1952). Role of fluoride ion in release of phosphate adsorbed by aluminum and iron hydroxides. Soil Science. 74:141-148.

Tyner, E.H. and J.G. Davide (1962). Some criteria for evaluating phosphorus tests for low-land rice soils. In: Trans. Comm. IV and V Int. Soc. of Soil Science. New Zealand Soil Bureau, New Zealand pp. 625-634.

Van Der Zee, S.E.A.T.M., M.M. Nederlof, W.H. Van Riemsdijk and F.A.M.d. Haan (1988). Spatial variability of phosphate adsorption parameters. Journal of Environmental Quality. 17:682-688.

Van Der Zee, S.E.A.T.M. and W.H. Van Riemsdijk (1986). Sorption kinetics and transport of phosphate in sandy soil. Geoderma. 38:293-309.

Van Der Zee, S.E.A.T.M. and W.H. Van Riemsdijk (1988). Model for long-term phosphate reaction kinetics in soil. Journal of Environmental Quality. 17:35-41.

Van Lierop, W. (1988). Determination of available phosphorus in acid and calcareous soils with the Kelowna multiple-element extractant. Soil Science. 146:284-291.

Van Lierop, W. and T.S. Tran (1990). Relationship between crop response and available phosphorus by Kelowna and EDTA and DPTA-modified multiple-element extractants. Soil Science. 149:331-338.

Vander-Zaag, P., R.L. Fox, R. De-La-Pena, W.M. Laughlin, A. Ryskamp, S. Villagarcia and D.T. Westermann (1979). The utility of phosphate sorption curves for transferring soil management information. Tropical Agriculture. 56:153-160.

Veith, J.A. and G. Sposito (1977). Reactions of aluminosilicates, aluminium hydrous oxides, and aluminium oxide with o-phosphate: the formation of X-ray amorphous analogs of variscite and montebrasite. Soil Science Society of America Journal. 41:870-876.

Walmsley, D. and I.S. Cornforth (1973). Methods of measuring available nutrients in West Indian soils II. Phosphorus. Plant and soil. 39:93-101.

Webber, M.D. and G.E.G. Mattingly (1970). Inorganic soil phosphorus. I. Changes in monocalcium phosphate potentials on cropping. Journal of Soil Science. 21:111-120.

White, R.E. (1980). Retention and release of phosphate by soil and soil constituents In: Soils and Agriculture. Critical Reports on Applied Chemistry. Volume 2. Society of Chemical Industry, Oxford, Blackwell Scientific Publications pp. 71-114.

White, R.E. (1966). Studies on the phosphate potentials of soils. IV. The mechanism of the soil:solution ratio effect. Australian Journal of Soil Research. 4:77-85.

White, R.E. and A.W. Taylor (1977). Reactions of soluble phosphate with acid soils: The interpretation of adsorption-desorption isotherms. Journal of Soil Science. 28:314-328.

Wild, A. (1988). Plant nutrients in soil: Phosphate. In: Russell's Soil Conditions and Plant Growth (11th edition). Longman Group UK Ltd pp. 695-742.

William, B.L. and G.P. Sparling (1984). Extractable N and P in relation to microbial biomass in UK acid organic soils. Plant and soil. 76:139-148.

Williams, E.G. and A.H. Knight (1963). Evaluation of soil phosphate status by pot experiments, conventional extraction methods, and labile phosphate values estimated with the aid of phosphorus-32. Journal of Science Food and Agriculture. 14:555-563.

Williams, J.D.H., J.K. Syers, R.F. Harris and D.E. Armstrong (1971). Fractionation of inorganic phosphate in calcareous lake sediments. Soil Science Society of America Proceedings. 35:250-255.

Williams, J.D.H., J.K. Syers and T.W. Walker (1967). Fractionation of soil inorganic phosphate by modification of Chang and Jackson's procedure. Soil Science Society of America Proceedings. 31:736-739.

Wolf, D.C., T.H. Dao and H.D.Scott. (1989). Influence of sterilization methods on selected soil microbiological, physical and chemical properties. Journal of Environmental Quality. 18:39-44.

Woodruff, J.R. and E.J. Kamprath (1965). Phosphorus adsorption maximum as measured by the Langmuir isotherm and its relationship to phosphorus availability. Soil Science Society of America Proceedings. 29:148-151.

Yee, A.R. and K. Broersma (1987). The Bray, Mehlich and Kelowna Soil P tests as affected by soil carbonates. Canadian Journal of Soil Science. 67:399-404.

Yerokun, O.A. (1987). Characterization of residual phosphorus in some Michigan soils. Ph.D. thesis, Michigan State University.

Yong, S.L. and R.J. Bartlett (1977). Assessing phosphorus fertilizer needs based on inetensity-capacity relationships. Soil Science Society of America Journal. 41:710-712.

Zhang, G.Y., X.N. Zhang and T.R. Yu (1987). Adsorption of sulphate and fluoride by variable charge soils. Journal of Soil Science. 38:29-38.